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INDUCTION OF GASTRIC TUMORS IN STRAIN A MICE BY METHYLCHOLANTHRENE

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Reports of the production of tumors of the stomach in experimental animals are so uncommon that their number is out of all proportion to the importance and frequency of this lesion as it occurs in man.

Considering the large number of animals that have been subjected to experimental tarring or painting with pure carcinogenic chemicals, the development of gastric neoplasms has been extremely rare. The Tworts [1] stated that among 60,000 mice painted with tar small papillomas of the stomach were observed only occasionally. After examining the stomachs of several hundred tarred mice, Bonné [2] found papillomas in a few animals which had survived two hundred days and in 1 animal a squamous cell carcinoma of the forestomach. Shabad [3] also reported the production of a benign papillomatous tumor in the forestomach of a mouse in a group that had received tar by rectal injection. Twort and Bottomley [4] reported a squamous cell epithelioma in the forestomach of a mouse whose skin had been painted for twenty weeks with chrysene ammonium and sodium sulfonate; they seem to have believed it a spontaneous tumor, since in 12,000 mice with induced cutaneous tumors this was the only tumor of a mucous membrane. On the other hand, the belief has been expressed that such neoplasms of the alimentary canal may result from ingestion of the carcinogenic chemical by the animal's licking the painted area or consuming contaminated food. However, this would not explain the gastric papilloma and carcinoma in female mice painted with 1,2,5,6-dibenzanthracene combined with theelin, reported by Perry and Ginzton,[5] since tumors of the stomach have not been found to be associated with uncomplicated painting of the skin with 1,2,5,6-dibenzanthracene in other laboratories where this procedure is common practice.

From National Cancer Institute, United States Public Health Service.

1. Twort, J. M., and Twort, C. C.: J. Path. & Bact. **35**:219, 1932.
2. Bonné, C.: Ztschr. f. Krebsforsch. **25**:1, 1927.
3. Shabad, L. M.: Vestnik rentgen. i radiol. **6**:223, 1928.
4. Twort, C. C., and Bottomley, A. C.: Lancet **2**:776, 1932.
5. Perry, I. H., and Ginzton, L. L.: Am. J. Cancer **29**:680, 1937

The injection of carcinogenic agents into the wall of the stomach and the oral administration of these substances in a deliberate attempt to provoke gastric tumors have yielded positive results in some cases and negative results in others. Uehlinger and Schürch [6] introduced a mixture of 0.005 mg. of mesothorium in petrolatum into the gastric wall in 8 rabbits, 2 of which died twenty-one months and twenty-nine months later with sarcoma of the stomach. Ilfeld [7] inserted pellets of benzpyrene under the gastric mucosa in 6 ferrets and pellets of 1,2,5,6-dibenzanthracene into the gastric wall of a dog, with negative results after a year. Fibiger [8] found that carcinoma of the stomach developed in mice, as well as in rats, when they were infected with spiroptera. However, this work has recently failed of confirmation by Cramer.[9] There is a case mentioned by Creighton [10] as having been reported by Twort, in which a cancer of the pylorus of a mouse apparently resulted from injury produced by a piece of swallowed glass. A papillary carcinoma in the forestomach of a male mouse was reported by Mercier and Gosselin [11] as following an intraperitoneal injection of tar in olive oil. Voronoff and Alexandrescu [12] administered a mixture of tar, hydrous wool fat, aniline oil and toluylenediamine by mouth to 10 rats and observed the development of carcinoma of the stomach in 1. Tani [13] found tumor-like changes resembling carcinomatous areas in the wall of the forestomach in mice receiving tar by mouth. Roffo [14] found that ingestion of fats that had been oxidized by heating and added to the ordinary food (bread and milk) of the white rat regularly provoked in these animals ulcer, papilloma and adenocarcinoma of the cardiac and pyloric chambers of the stomach during an experimental period of two years. He ascribed the cancer-producing role of these fats to the formation of oxycholesterol. He [15] also reported that in the rat ingestion of a diet containing cholesterol irradiated by the sun or with ultraviolet rays results in gastric adenocarcinoma. Waterman [16] found that cholesterol oleate administered orally alone, without any addition of known carcinogenic substance, produced malignant changes in the stomach. In 4 animals infiltrating papilloma with hyperkeratosis was produced and

6. Uehlinger, E., and Schürch, O.: Deutsche Ztschr. f. Chir. **251**:12, 1938.
7. Ilfeld, F. W.: Am. J. Cancer **26**:743, 1936.
8. Fibiger, J.: J. Cancer Research **4**:367, 1919.
9. Cramer, W.: Am. J. Cancer **31**:537, 1937.
10. Creighton, C.: Some Conclusions on Cancer, London, Williams & Norgate, Ltd., 1920, p. 120.
11. Mercier, L., and Gosselin, L.: Compt. rend. Soc. de biol. **113**:669, 1933.
12. Voronoff, S., and Alexandrescu, G.: Néoplasmes **8**:129, 1929.
13. Tani, I.: Tr. Jap. Path. Soc. **21**:715, 1931.
14. Roffo, A. H.: Bol. Inst. de med. exper. para el estud. y trat. d. cáncer **48**: 407, 1938.
15. Roffo, A. H.: Bol. Inst. de med. exper. para el estud. y trat. d. cáncer **46**: 589, 1938.

in 1 true adenocarcinoma of the stomach. Waterman [16] also fed a 0.4 per cent solution of benzpyrene in lard to 6 mice, several milligrams being consumed daily by each animal. In 5 animals squamous cell carcinoma of the stomach developed in from one hundred and twelve to three hundred and thirty-six days, and in 4 of these there were metastases to the lymph nodes, peritoneum, liver, spleen or lung. Van Prohaska, Brunschwig and Wilson [17] found that, of 48 mice receiving methylcholanthrene orally and observed for periods ranging up to one hundred and eighty-six days, 2 presented benign squamous epithelial papilloma of the fundus of the stomach. There have been a number of reports of negative attempts to produce cancer of the stomach by oral administration of carcinogenic hydrocarbons.[18] The experiments reported by Kinosita [19] on the development of gastric tumors in rats receiving butter yellow, tetramethyldiaminobenzophenone and aminoazobenzene orally have not been sufficiently controlled to rule out the possibility of vitamin A deficiency as an etiologic factor in the production of the lesions described by him.

EXPERIMENTAL PROCEDURE

Thirty strain A male mice aged 3 months were employed in this experiment. The mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. They were maintained on a diet of dog chow [20] exclusively except for the first few days after being received in this laboratory, during which time the diet was supplemented with bread and milk. Tap water was allowed freely at all times. When a mouse was to be given an injection it was anesthetized with pentobarbital sodium, and the stomach was exposed through a midline abdominal incision. The stomach of the mouse is composed of two chambers, sharply demarcated from each other on both the internal and the external surface of the viscus. The left, or cardiac, chamber is lined by squamous epithelium directly continuous with that of the esophagus. The pyloric chamber, which is continuous with the cardiac chamber, is lined by glandular epithelium, similar to that of other mammals, and terminates in the duodenum. A solution of methylcholanthrene in liquid petrolatum was injected into the anterior wall of either the cardiac or pyloric chamber or both. The solution contained 10 mg. of methylcholanthrene per cubic centimeter of liquid petrolatum and was injected in the dose of 0.03 to 0.05 cc. The solution became clear when heated to 40 C., at which temperature it was

16. Waterman, N.: Acta cancrol. **2**:375, 1936.

17. van Prohaska, J.; Brunschwig, A., and Wilson, H.: Arch. Surg. **38**:328, 1939.

18. (*a*) Reinhard, M. C., and Candee, C. F.: Am. J. Cancer **26**:552, 1936. (*b*) Oberling, C.; Sannié, C.; Guérin, M., and Guérin, P.: Bull. Assoc. franç. p. l'étude du cancer **25**:156, 1936. (*c*) Cook, J. W.; Haslewood, G. A. D.; Hewett, C. L.; Hieger, I.; Kennaway, E. L., and Mayneord, W. V.: Am. J. Cancer **29**: 219, 1937. (*d*) Lorenz, E., and Stewart, H. L.: Unpublished data. (*e*) Waterman.[16] (*f*) van Prohaska and others.[17]

19. Kinosita, R.: Tr. Jap. Path. Soc. **27**:665, 1937.

20. According to the Purina Mills Company, the chow contains the following ingredients: protein, 20 per cent; fat, 3 per cent; carbohydrate, 56 per cent; ash, 6 per cent, and water, 15 per cent, with vitamins A and G added.

injected. The clinical course of most of the animals remained satisfactory throughout the experiment. In some animals palpable inflammatory masses developed in the upper part of the abdomen over the stomach. The survival periods were as follows: 1, 1, 7, 6, 3, 4, 2 and 3 mice lived for six, eight, ten, twelve, thirteen, fourteen, sixteen and seventeen months, respectively; 3 died postoperatively. All the mice were examined post mortem except 1 mouse which was partially eaten by its cage mates.

OBSERVATIONS

In 4 animals squamous cell carcinoma of the stomach developed, and in 4, squamous papilloma of the stomach. The 4 mice with carcinoma of the stomach were submitted to autopsy ten, fourteen, fourteen and seventeen months, respectively, after the injection of the carcinogen; the 4 mice with papilloma of the stomach, eleven, thirteen, sixteen and sixteen months after treatment, respectively. In addition to the tumors, the following lesions were noted in different animals at autopsy: hyperplasia of the gastric mucous membrane; chronic peritonitis with adhesions in the upper part of the abdomen; multiple adenomatous tumors of the lung; chronic nephritis; lymphoma; abscess of the liver, spleen and prostate gland; cirrhosis; amyloidosis; chronic pancreatitis; pyloric obstruction; hemorrhage into the colon and stomach, and ulcerative dermatitis.

No tumors developed in 13 control mice with pieces of untreated yarn soaked in lard or in liquid petrolatum or with plain cotton thread stitched into the wall of the stomach.

Papilloma.—There were 4 cases of papilloma of the stomach. Two of the tumors were solitary small gray nodules, 4 mm. and 6 mm. in diameter, respectively, each projecting into the lumen of the cardiac chamber of the stomach (fig. 1*A*). The other 2 lesions were small and scarlike, and involved the mucous surface of the stomach. The latter tumors were indistinguishable grossly from the scarring and thickening of the gastric wall which occurred occasionally as a result of inflammatory changes evoked by the presence of the hydrocarbon.

Microscopic examination revealed that in 3 of the cases the lesion was located at the junction of the cardiac and pyloric chambers, while in the other case it was confined to the cardiac chamber. Structurally each tumor consisted of a central stalk of connective tissue covered with squamous epithelium. In 3 cases the stalk supporting the papilloma was broad and in the other case narrow and high. The surfaces of the flat lesions were studded with spinelike projecting papillae. The covering cells were fairly regular, well differentiated stratified squamous epithelium. Mitotic figures were infrequent in 3 cases, and in the fourth case from two to four were observed in several single high power fields. The epithelial covering of the tumor merged abruptly with the mucous membrane of the stomach on either side of the lesion, without any marked hyperplasia of the cells at the zone of transition but with slight hyperkeratosis in this region in 1 case. The connective tissue

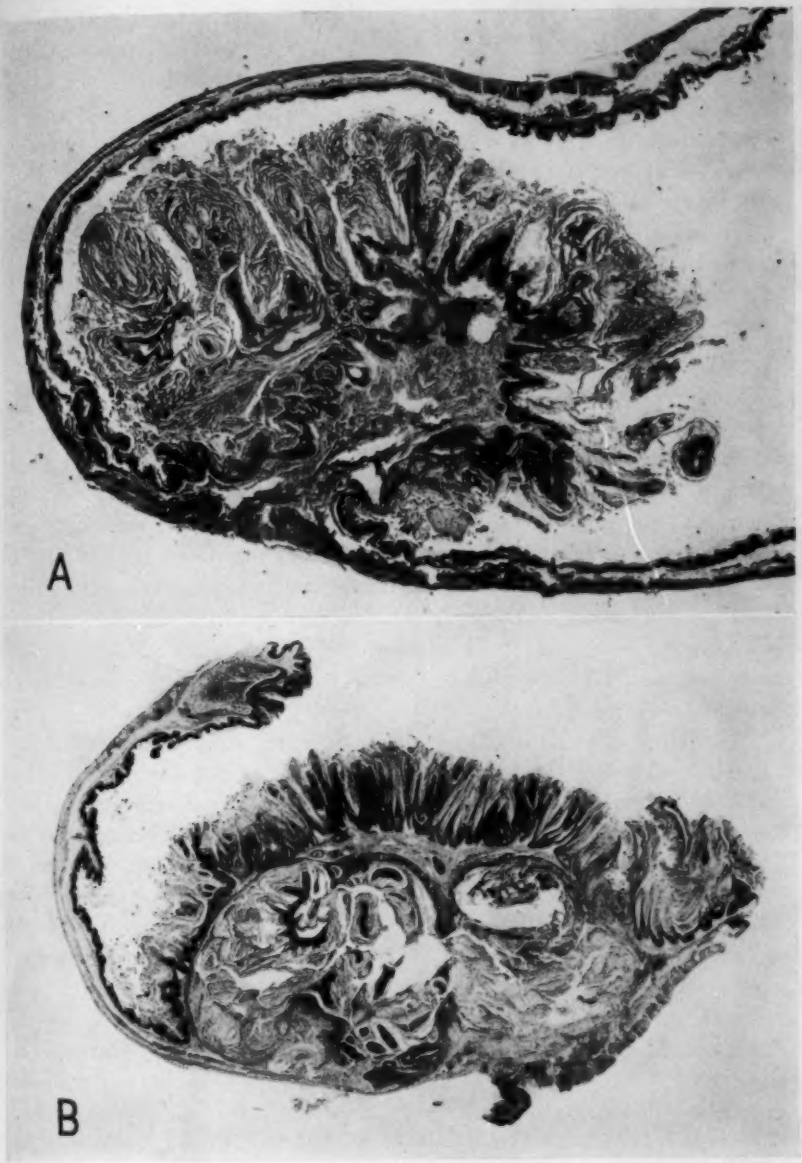

Fig. 1.—*A*, squamous papilloma of the cardiac chamber of the mouse stomach produced by injecting a solution of methylcholanthrene in liquid petrolatum into the wall of the stomach sixteen months previously; × 25. *B*, portion of the cardiac chamber of the stomach of a mouse, showing papillary squamous cell carcinoma; × 10. The mouse had received an injection of methylcholanthrene in liquid petrolatum into the wall of the stomach seventeen months previously.

stroma was composed of adult fibroblasts with a few well formed blood vessels and in 1 case numerous inflammatory cells. The smooth line of demarcation between the epithelium and the underlying stroma, which is characteristic of a papilloma, was not always maintained. On the contrary, there was a tendency in all specimens for masses and strands of squamous cells to penetrate, in limited numbers, the underlying stroma, becoming apparently separated from the surface cells. In 3 cases this cellular infiltration did not go beyond the basement membrane of the gastric mucosa; in 1 case there was a small epithelium-lined cyst in the submucosa, adjacent to the muscular wall of the stomach.

Carcinoma.—There were 4 cases of carcinoma of the stomach. The lesions measured from 5 to 9 mm. in diameter. On cross section, each consisted of a round gray nodule elevated 5 mm. or so above the surface level of the mucous membrane of the cardiac chamber (fig. 1 *B*), and all but 1 involved the anterior wall of the viscus. The latter was situated on the posterior inferior margin some little distance from the point of injection. Three lesions had finely granular surfaces; the fourth was a crater-like ulcer, covered with clotted blood, and the lumen of the viscus was filled with blood. In 2 cases the tumor on the mucous surface of the stomach was continuous with a rounded, umbilicated lesion on the peritoneal surface. The surrounding gastric wall was puckered and retracted on all sides toward the peritoneal extension of the tumor. In 1 case a portion of the liver adherent to the serosa of the stomach opposite the tumor contained a small abscess.

Microscopically, the malignant tumors were papillary squamous cell carcinoma, showing extensive keratinization of the covering epithelium. Each was located in the cardiac chamber. The gastric mucous membrane at its junction with the lesion was hyperplastic and hyperkeratotic. One tumor was ulcerated, with the base of the ulcer situated at the level of the circular musculature. The lumen of the stomach in this case was filled with blood. Every lesion exhibited frank infiltration of tumor cells into all the coats of the viscus. The infiltrating epithelium was composed of basal cells, prickle cells and flat squamous cells, which were atypical in size, shape and staining and showed numerous mitotic figures (fig. 2 *A*). The centers of the cellular masses frequently contained concentric layers of keratin, but in 1 tumor some of them were empty, suggesting an alveolar structure with a squamous lining. In 1 case there were nests of epithelium within thin-walled vessels, either lymph vessels or blood vessels, situated between the two muscular layers of the gastric wall (fig. 2 *B*). The stroma of all the tumors was infiltrated with inflammatory cells, which were especially numerous along the course of the permeating tumor cells. In the case in which the liver was abscessed and adherent to the wall of the stomach opposite the

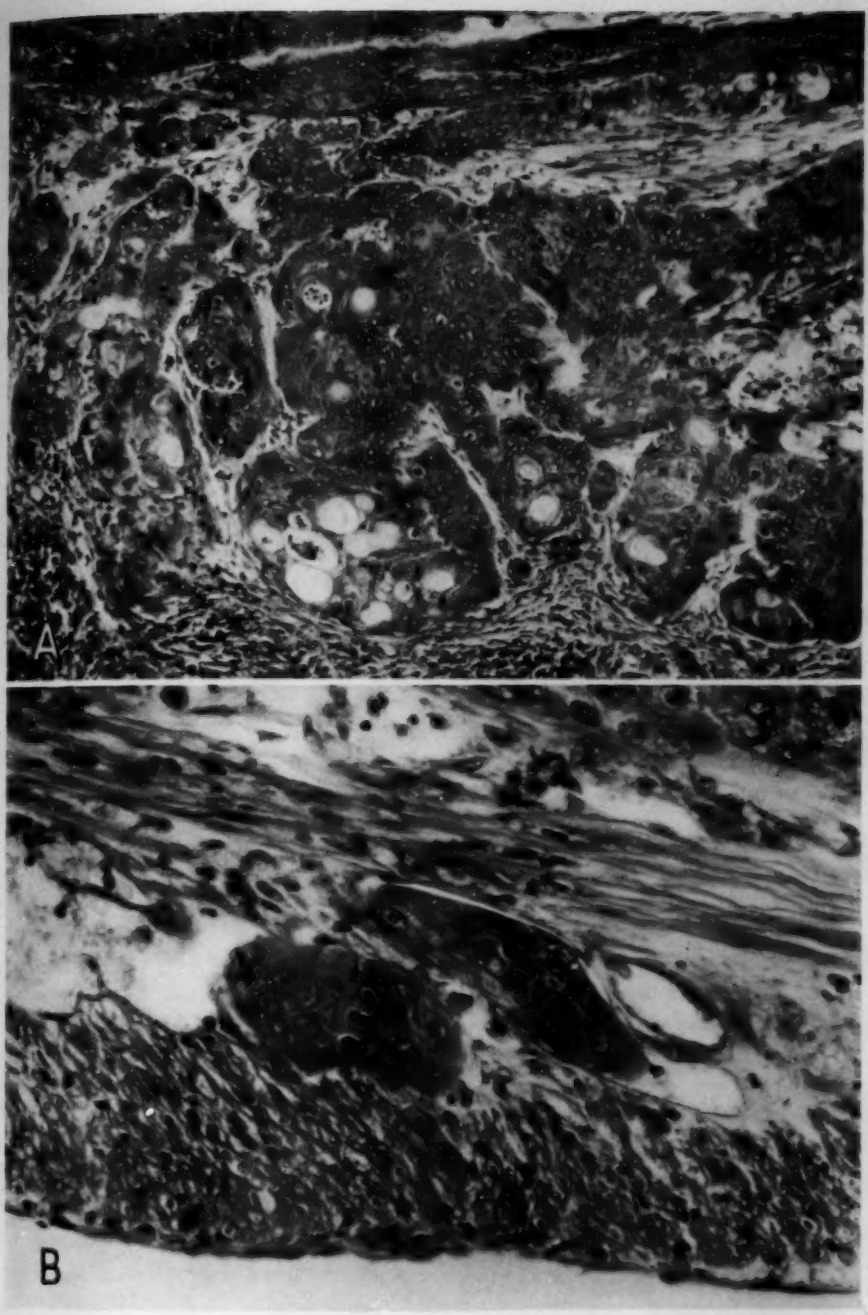

Fig. 2.—*A*, an area of the tumor illustrated in figure 1 *B*; × 140. In this field there are masses and cords of atypical squamous epithelium infiltrating the wall of the stomach. *B*, the muscular wall of the gastric tumor shown in figure 1 *B* and figure 2 *A*; × 400. The peritoneal surface is visible in the lower portion of the illustration. Between the muscle coats are two isolated masses of tumor cells within thin-walled vessels.

carcinoma there were masses of keratin in the center of the abscess. In 2 cases small nodules composed of tumor cells were adherent to the external surface of the peritoneum.

In 1 case bits of tissue from the squamous cell carcinoma of the stomach were transplanted into 6 male strain A mice; the transplants were inserted subcutaneously in the axilla in 3 animals and into the peritoneal cavity in 3 animals. Three weeks later 1 mouse with an axillary transplant and 3 mice with intraperitoneal transplants showed large tumors at the sites of inoculation. Transplants were then made from these inoculated tumors into corresponding locations in 6 strain A mice, and large tumors developed in 2 of these within one month. All the tumors that developed from the transplants were squamous cell carcinoma, morphologically identical with the original tumor of the stomach from which they were obtained.

COMMENT

The results of this experiment seem to indicate that the mucous membrane of the stomach of the mouse, although susceptible to experimental induction of tumors, is relatively resistant to the carcinogenic action of methylcholanthrene as compared with other body tissues similarly treated on which data are available for comparison. In only 8 of the 30 mice given injections in this experiment did tumors of the stomach develop. It was not possible to determine accurately the latent period of tumor development, because the stomach was not inspected directly and abdominal palpation was of direct aid in determining the onset of tumor. However, a rough estimate of the time required for the lesions to attain the proportions noted at the postmortem examination may be gained from a consideration of the period which elapsed between the injection of the agent and the autopsy on the animal. This period averaged fourteen months both for papilloma and for carcinoma. This is considerably longer than the average time required for bulkier cutaneous or subcutaneous tumors to develop in mice of this strain treated with equivalent doses of methylcholanthrene. Additional evidence indicating the relatively greater resistance of the gastric epithelium of the mouse to induction of tumors was obtained in another experiment in which pieces of yarn soaked in a 5 per cent solution of methylcholanthrene in lard were stitched into the wall of the stomach in 14 C_3H mice, in none of which did gastric tumors develop although 7 animals survived the experimental procedure by ten to seventeen months. In the present experiment, although the solution of methylcholanthrene in liquid petrolatum was injected into the wall of both the cardiac and the glandular chamber, the tumor arose only in the cardiac chamber, and in each instance it was of the squamous cell type, thus

indicating still greater resistance on the part of the glandular mucous membrane of the stomach to the carcinogenic effects of methylcholanthrene as compared with the mucosa of the forestomach.

The negative results from many attempts to produce gastric tumors by feeding carcinogenic hydrocarbons has been thought to depend on the total insusceptibility of the gastric epithelium to the action of these agents or on the lack of absorption or on a chemical change occurring as a result of digestion. However, certain findings show that the resistance to the induction of tumors of the gastrointestinal tract by oral administration of 1, 2, 5, 6-dibenzanthracene to mice is not due to lack of absorption or chemical alteration. Lorenz and Stewart [18d] showed that pulmonary tumors develop in mice ingesting 1, 2, 5, 6-dibenzanthracene, indicating that this agent is absorbed in sufficient amounts to exert a carcinogenic action on lung tissue; furthermore, the hydrocarbon was found unchanged, in large part at least, in the alimentary canal up to the level of the ileocecal valve. That the gastric mucous membrane is not totally insusceptible to the carcinogenic action of methylcholanthrene and benzpyrene when these substances are administered orally is proved by the positive results obtained by Waterman [16] and van Prohaska, Brunschwig and Wilson.[17] The present experiment also shows that the resistance of the gastric mucous membrane of the forestomach to the induction of tumors is only a relative matter.

From the morphologic description of the lesions obtained in this experiment, it is apparent that the division of the tumors into papilloma and carcinoma was an arbitrary one for the purpose of classification. This division was made on the basis of the presence or absence of invasion of the deep muscular coats of the stomach by tumor cells. Such invasion was present in the lesions diagnosed as carcinoma, and it was absent in the specimens of papilloma. However, in the specimens of papilloma the smooth line of demarcation between the surface epithelium and the underlying stroma was not always maintained. Instead all these specimens exhibited to some extent excessive growth of epithelium with extension of tumor cells through this barrier into the underlying stroma in the form of masses and strands of cells apparently detached from the surface epithelium. In 1 case there was a mass of atypical epithelium below the basement membrane, in the submucosa. These findings suggest that under the conditions of this experiment transition stages may occur between papilloma of the stomach and carcinoma similar to the development of squamous cell epithelioma from benign warts of the skin on an area of the epidermis subjected to applications of a carcinogenic agent.

Of the attempts to produce experimental tumor of the stomach by injection of a carcinogenic agent into the wall of the stomach as reviewed in the introduction to this paper there are two which have a bearing

on the present experiments. Ilfeld [7] inserted pellets of benzpyrene under the gastric mucosa of 6 ferrets and 1, 2, 5, 6-dibenzanthracene pellets into the gastric wall of a dog, with negative results after a year. Nothing is known at present regarding the latent period of induction of tumor in the ferret, but a recent study by Passey [21] has shown that in the dog it requires several years to evoke tumors of the skin by painting with a carcinogenic tar. It therefore seems that the period of observation in Ilfeld's experiment might not have been long enough for positive results to be obtained. Uehlinger and Schürch [6] obtained sarcoma of the stomach in 2 of 8 rabbits following implantation of a mixture of mesothorium in petrolatum into the stomach wall. It is interesting that in the present experiment, although the carcinogen was likewise injected into the muscular and connective tissue of the wall of the stomach, the tumors which developed were epithelial in type.

Consideration has been given the possibility that the gastric tumors obtained in the present experiment were spontaneous in origin. This possibility is unlikely, for spontaneous carcinoma of the stomach in mice is extremely rare. Wells, Slye and Holmes [22] reported that in 142,000 mice of the Slye stock, all dying of natural causes and most of them of cancer age, without experimental manipulation, only 15 were found to have a primary malignant neoplasm of the stomach, 8 having squamous cell carcinoma of the cardia, 3 adenocarcinoma of the pylorus, 3 apparently benign epithelioma and 1 primary sarcoma. Reports of only 8 other cases of spontaneous gastric cancer in mice have been found in the literature (Stewart and Andervont [23]; Wells, Slye and Holmes [22]). Of many thousands of mice of different strains and ages, many of them subjected to various experimental procedures employed in this laboratory, which have been examined post mortem during the past several years, not a mouse has shown a spontaneous or an induced malignant tumor of the stomach. Stewart and Andervont described an unusual type of adenomatous hyperplasia of the gastric mucous membrane occurring regularly in mice of strain I, but this lesion involves the glandular mucous membrane and is entirely different structurally from the induced tumors of the squamous mucosa of the forestomach dealt with in the present report.

SUMMARY

Squamous papilloma of the stomach was produced in 4 mice and squamous cell carcinoma of the stomach in 4 mice of strain A by injection of a solution of methylcholanthrene in liquid petrolatum into the anterior wall of the stomach of either or of both gastric chambers.

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STUDIES ON RESISTANCE TO TRANSMISSIBLE LEUKEMIA IN MICE BY MEANS OF PARABIOSIS

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Parabiosis offers an excellent means of investigating the role of humoral factors in resistance and susceptibility to transmissible neoplasms. Several investigators have already used this method in a search for circulating antibodies in the serums of laboratory animals resistant to transmissible neoplasms, but their results were contradictory. Transmissible leukemia of mice, a neoplasm of the blood-forming organs, is fatal after a definite duration of illness, characteristic for each strain. This permits a relatively accurate determination of factors that may modify the course of leukemia. We have undertaken a study of the hypothetic factors of resistance and susceptibility to leukemia by the method of parabiosis.

In 1863 Paul Bert, an anatomist, studying the problem of transplantation, united two rats by suturing together their skin, muscle and peritoneum (quoted by Møller-Christensen [1]). In 1908 Sauerbruch and Heyde [2] described an operation designed to make a permanent union between two animals by celioanastomosis, and named the condition resulting from this operation parabiosis. Rats were found to be most suitable, but guinea pigs, mice, rabbits, monkeys and other animals were also successfully united. Bunster and Meyer [3] modified Sauerbruch's technic by suturing the four leaves of the peritoneum together, thus eliminating a common peritoneal cavity and excluding herniation of intestinal contents from one animal to the other.

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2. Sauerbruch, F., and Heyde, M.: München. med. Wchnschr. **55**:153, 1908.

3. Bunster, M., and Meyer, R. K.: Anat. Rec. **57**:339, 1933.

Parabiosis has been used in studies of metabolism, immunity to infection, resistance to tumors and, more recently, hormones (Møller-Christensen [1]).

Several investigators have studied the problem of immunity to tumors by means of parabiosis, but the results are not uniform. Rous [4] united tumor-bearing rats with rats that proved resistant to three successive injections of the same tumor and noted no effect on the tumor growth. The results of Rous were confirmed by Morpurgo,[5] who transplanted sarcoma to susceptible rats that were connected with resistant ones. The resistant rats did not exert any influence on the tumor growth. Albrecht and Hecht [6] transplanted tumor into one of parabionts and found that the tumor grew more slowly in the parabiont than in single mice. They concluded from their experiments that the normal parabiont inhibited growth of the tumor transplanted in the parabiotic host. If they separated the mice, the tumor grew more rapidly. Lambert [7] united rats that were inoculated with mouse tumor with normal mice and found that the growth of the mouse tumor in the rats was promoted by this union. He assumed that this was brought about by passage of nutritive material from the mouse to the rat. Recently Fischer [8] united mice bearing spontaneous tumors with normal mice and observed acceleration of the tumor growth.

MATERIAL AND METHODS

The mice used in this study were members of stocks A, R and S, which have been inbred in this laboratory since 1928.[9] Mice of stock C were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The susceptibility of stocks A and R to different strains of leukemia has been established through numerous experiments. Mice of stock R are resistant to leukemia that originates in stock A but susceptible to leukemia appearing in stock R, and vice versa. Stock S is partially susceptible to leukemia occurring in stock A, and vice versa.

The interval between inoculation of leukemic cells and death from leukemia varies with different strains of leukemia. Most of the transmissible strains of lymphoid leukemia used in this study caused death of single susceptible animals approximately one to three weeks after inoculation. The myeloid and monocytic strains had a more chronic course. Parabiotic and control mice received, in the

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tail vein, 0.1 cc. of a suspension of leukemic cells in Tyrode's solution, obtained usually from the spleen of a leukemic mouse. This suspension contained approximately 10,000 cells per cubic millimeter. Mice that died of diseases other than leukemia before the expected time of death from leukemia were omitted from the tables. Usually mice of the same sex, approximately 6 to 12 weeks of age, were united. In many instances, when one mouse died its parabiont was separated surgically and subsequently observed for a period of from several days to two months.

The technic used was that of Sauerbruch[2] as later modified by Bunster and Meyer.[3] Briefly, it consists in union of two animals by means of peritoneum, abdominal muscles and skin, with scapulas sutured together for firmer support.

In preliminary experiments it became apparent that shortly after operation the mice began to strain and tear the cephalad end of the suture line. In order to prevent this, a neckband of aluminum, measuring approximately 8 by 0.3 by 0.1 cm., partly covered with adhesive tape, was fitted around the neck of each mouse and joined at the time of operation.

EXPERIMENTS

The experiments will be described in four groups. In group 1 two mice susceptible to leukemia were united. In group 2 a susceptible and a resistant mouse were joined, and the susceptible mouse was inoculated with leukemic cells. In group 3, also consisting of paired susceptible and resistant animals, the resistant animal was inoculated. In group 4, susceptible mice were united with mice of a stock that was partially resistant to the strain of leukemia used; in some instances the partially resistant, in others the susceptible mouse, was inoculated.

Inoculation of Parabiotic Twins Both of Which Were Susceptible to Leukemia.—Since a tumor graft is influenced by the general health of the animal, it seemed desirable to determine what effect, if any, union with another susceptible mouse would have on the course of leukemia in an inoculated susceptible animal as compared with an inoculated single mouse. This series of experiments would show also whether leukemic cells pass from the inoculated susceptible to the uninoculated susceptible mouse.

The results of the injection of leukemic cells from four different strains into one of two susceptible parabionts are shown in table 1. In 8 of the 9 parabiotic twins, both mice died of leukemia. The leukemic infiltrations were almost as advanced in the uninoculated as in the inoculated animal. The average length of life after inoculation of the parabiotic mice was approximately the same as that of single control mice given similar injections.

Inoculation of Parabiotic Twins of Which One Mouse Was Susceptible, the Other Resistant.—(*a*) Inoculation of the Susceptible Mouse: These experiments were performed to determine whether the inhibiting factors present in the resistant animal would pass from the resistant uninoculated animal to the susceptible inoculated one,

thereby either preventing or delaying the development of leukemia. The susceptible mice were inoculated intravenously from one to twenty-seven days before and from three to seven days after parabiosis

TABLE 1.—*Inoculation of Susceptible Mouse in Parabiosis with Susceptible Mouse* *

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected†	Days Between Operation for Parabiosis and Injection	Result of Inoculation		Controls			
				Inoculated Mouse	Uninoculated Mouse	Number Inoculated	Number Showing Positive Result	Days of Life After Inoculation	
								Extremes	Average
26	C.R♂-C.R♂	Rfb 385	25	+ D 42	+ D 42	10	9	D 31-43	39
31	Ak.R♀-Ak.R♀	Rfb 385	25	+ D 28	+ D 28	4	2	D 22-33	27.5
33	Ak.R♀-Ak.R♀	Rfb 385	21	+ D 18	+ D 18	4	2	D 22-33	27.5
34	Ak.R♂-Ak.R♂	Ha 230	31	+ D 15	+ D 15	9	9	D 13-23	17
37	Ak.R♂-Ak.R♂	Ha 230	22	+ K 10	+ K 10	9	9	D 13-23	17
39	Ak.R♀-Ak.R♀	Ha 230	18	+ D 17	+ D 17	9	9	D 13-23	17
42	Ak.R♀-Ak.R♀	Hb 4	20	+ D 26	+ D 23	4	3	D 24-26	25
45	Ak.R♀-Ak.R♀	Hb 4	14	+ D 24	+ D 24	4	3	D 24-26	25
21§	S♂-S♂	Slb 351	35	— K 113	— D 96	2	2	D 15-16	15.5

* In all the tables the abbreviations used are as follows: C.R, hybrid mice between C and R mice; Ak.R, hybrid mice between Ak and R mice; Ak-R, Ak mouse in parabiosis with R mouse; +, inoculation successful; —, inoculation unsuccessful; D, died; K, killed. The figures following D and K indicate the number of days after inoculation.

† Strain Rfb 385 is monocytic, Ha 230 and Hb 4 are lymphoid, and Slb 351 is myeloid (chloroleukemia).

§ This parabiont inoculated with chloroleukemia Slb 351 failed to show the disease, although both control mice succumbed to it. This strain of chloroleukemia yields variable results and in occasional experiments inoculated mice remain healthy.

TABLE 2.—*Inoculation of Susceptible Mouse in Parabiosis with Resistant Mouse*

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected	Days Between Injection and Operation for Parabiosis	Result of Inoculation	
				Inoculated Susceptible Mouse, Days After Inoculation	Uninoculated Resistant Mouse, Days After Operation
109	Ak.R♀-R♀	Akf 5*	1	— D 13	— K 13
103	Ak.R♀-R♀	Akf 5	2	+ D 13	— D 13
104	Ak.R♀-R♀	Akf 5	2	+ D 11	— K 48
155	Ak.R♂-R♂	Akh 106†	24	+ K 39	— K 64
157	Ak.R♂-R♂	Akh 106	25	+ K 52	— K 39
181	Ak.R♀-R♀	Akh 106	25	+ D 69	— K 67
182	Ak.R♀-R♀	Akh 106	27	+ K 41	— K 41
			Days Between Operation and Injection		
133	Af♂-Ak.R♂	Akf 5	3	+ D 9	— K 70
130	Af♂-Ak.R♂	Akf 5	7	+ D 7	— K 70

* Strain Akf 5 is acute lymphoid leukemia.

† Strain Akh 106 is chronic myeloid leukemia.

was effected. It was believed that if the susceptible mice were inoculated before union the leukemic cells would multiply and would be more numerous at the time of operation, so that a large number could pass to the resistant parabiont.

Of the 7 susceptible parabionts inoculated from one to twenty-seven days before operation, 6 died with advanced leukemia from eleven to sixty-nine days after inoculation. Both susceptible mice inoculated three and seven days after operation died with leukemia nine and seven days, respectively, after inoculation. In no uninoculated resistant mouse did leukemia develop.

In each experiment with lymphoid leukemia, from 3 to 6 normal susceptible mice were inoculated. All died with leukemia from ten to thirteen days after inoculation.

Of 7 mice inoculated with myeloid cells of strain Akh 106 (controls to twins 155 and 157), 6 were killed at an advanced stage of the disease, forty-three days after inoculation. In a later experiment all 8 mice inoculated (controls to twins 181 and 182) were killed and showed advanced leukemia twenty-seven days after inoculation.

TABLE 3.—*Inoculation of Resistant Mouse in Parabiosis with Susceptible Mouse*

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected	Days Between Operation for Parabiosis and Injection	Result of Inoculation	
				Inoculated Resistant Mouse, Days After Inoculation	Uninoculated Susceptible Mouse, Days After Operation
93	Af♀-Ak♀	Akf 5	5	— K 21	+ D 21
96	Af♀-Ak♀	Akf 5	3	— D 27	— K 27
127	Af♀-Ak.R♀	Akf 5	9	— K 15	— D 15
132	Af♂-Ak.R♂	Akf 5	5	— K 70	— K 70
135	Af♀-Ak.R♀	Akf 5	3	— K 70	+ D 28
129	Af♂-Ak.R♂	Akf 5	7	— K 46	— K 46
136	Af♀-Ak.R♀	Akf 5	2	— K 11	+ D 11
99	Ak.R♀-R♀	Akf 5	1	— D 20	— D 20
178	Ak.R♀-R♀	Akf 5	3	— K 19	+ K 19
179	Ak.R♀-R♀	Akf 5	3	— D 13	+ D 41
184	Ak.R♀-R♀	Akf 5	9	— K 36	— D 36

(*b*) Inoculation of the Resistant Mouse: The results summarized in table 3 show that in none of the resistant inoculated mice did leukemia subsequently develop whereas approximately one half of the uninoculated susceptible mice died of this disease from eleven to forty-one days after inoculation. Single susceptible mice inoculated with this strain died of leukemia from nine to eighteen days after inoculation.

The resistant inoculated mouse in pair 179 died thirteen days after inoculation. The susceptible parabiont was separated surgically and died of leukemia twenty-eight days after separation. The passage of malignant cells must have occurred within thirteen days after operation.

Previous experiments have shown that the length of life after inoculation is in inverse relation to the inoculating dose.[9b] The long and variable interval between inoculation of the resistant and death of the

uninoculated susceptible parabionts can be explained by assuming that most of the cells perished in the inoculated resistant mice and that only small variable numbers of leukemic cells entered the circulation of the susceptible mice.

TABLE 4.—*Inoculation of Mouse of Partially Resistant Stock in Parabiosis with Susceptible Mouse*

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected	Days Between Operation for Parabiosis and Injection	Result of Inoculation	
				Inoculated Mouse of Partially Resistant Stock	Uninoculated Mouse of Susceptible Stock
116	Ak.R♀-S♀	Akf 5	2	— D 14	— D 14
117	Ak.R♀-S♀	Akf 5	2	— D 13	— K 13
118	Ak.R♀-S♀	Akf 5	1	— D 12	— D 12
141	Ak.R♀-S♀	Akf 5	6	+ D 36	+ D 36
144	Ak.R♀-S♀	Akf 5	9	— K 53	+ D 18
146	Ak.R♀-S♀	Akf 5	2	+ D 18	+ D 18
147	Ak.R♀-S♀	Akf 5	2	+ D 13	+ D 13
150	Ak.R♀-S♀	Akf 5	40	— D 22	+ D 22
164	Ak.R♀-S♀	Akf 5	12	— D 51	+ D 51

TABLE 5.—*Inoculation of Susceptible Mouse in Parabiosis with Mouse of Partially Resistant Stock*

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected	Days Between Injection and Operation	Result of Inoculation	
				Inoculated Mouse of Susceptible Stock	Uninoculated Mouse of Partially Resistant Stock
108	Ak.R♀-S♀	Akf 5	Same day	+ D 13	— K 13
110	Ak.R♀-S♀	Akf 5	2	+ D 11	— K 48
121	Ak.R♀-S♀	Akf 5	3	+ D 10	— D 10
115	Ak.R♀-S♀	Akf 5	5	+ D 9	— K 9
159	Ak.R♀-S♀	Akh 106	22	+ D 38	— K 72
170	Ak.R♀-S♀	Akh 106	23	+ D 62	— K 58
167	Ak.R♀-S♀	Akh 106	34	+ D 77	— K 46
171	Ak.R♀-S♀	Akh 106	23	+ D 61	+ D 31
			Days Between Operation and Injection		
134	Ak.R♀-S♀	Akf 5	3	+ D 9	— K 70
137	Ak.R♀-S♀	Akf 5	2	+ D 9	— K 9
138	Ak.R♀-S♀	Akf 5	8	+ D 9	— K 9
142	Ak.R♀-S♀	Akf 5	6	+ D 13	— D 13

Inoculation of Parabiotic Twins of Which One Mouse Was from a Susceptible, the Other from a Partially Resistant Stock.—At the time when the Ak.R hybrids were paired with S mice we considered the S mice not susceptible to leukemia of stock Ak, but unexpectedly several of the inoculated S mice died of leukemia.

(*a*) Inoculation of Mouse of Partially Resistant Stock: The experiment included nine pairs of mice. Table 4 shows that the sus-

ceptible uninoculated parabionts of six pairs died of leukemia from thirteen to fifty-one days after inoculation of the partially resistant parabiont. Three of the inoculated, supposedly resistant parabionts also died of leukemia. In the remaining three pairs, 5 mice died and 1 was killed from twelve to fourteen days after inoculation and showed no evidence of leukemia.

The results of this experiment necessitated reinvestigation of the susceptibility of the S stock to lymphoid leukemia of stock Ak. Twenty-eight single mice of the S stock were inoculated, and 10 of these died of leukemia from nine to twenty-three days after inoculation.

The results of the experiments shown in table 4 indicate that of the S mice inoculated, some were susceptible and others were resistant to leukemia.

(*b*) Inoculation of the Susceptible Mouse: This group comprises twelve pairs of mice, in which 8 susceptible mice were inoculated before and 4 were inoculated after operation. Table 5 shows that all of the inoculated susceptible (Ak.R) mice died with leukemia but that with a single exception the uninoculated S mice remained healthy.

OBSERVATIONS ON THE CIRCULATION BETWEEN PARABIOTIC MICE

Numerous investigators have attempted to demonstrate that there is a communication of lymph and blood capillaries between parabionts (Møller-Christensen [1]; Rössle [10]; Mayeda [11]; Sauerbruch and Heyde [2]; Morpurgo.[12]) Sauerbruch and Heyde [2] injected iodine into a parabiotic rat and recovered it in the urine of the nontreated parabiont within forty-five minutes after the injection. Volumetric studies following intravenous or intracardiac injections of brilliant red (Hill [13]) showed that the dye is present in the same concentration in each of the parabiotic rats in approximately six hours. Immune bodies (Friedberger and Nasetti [14]) and bacteria (Sauerbruch and Heyde [2]; Ranzi and Ehrlich [15]; Steiner and Steinfeld [16]) pass from the parabiont into which dye was injected into the other parabiont. When india ink was injected into the heart of a parabiont (Goldman, quoted by Sauerbruch and Heyde [2]), both animals had deposits of black particles in liver and spleen.

10. Rössle, R.: Virchows Arch. f. path. Anat. **300**:31, 1937.
11. Mayeda, T.: Deutsche Ztschr. f. Chir. **167**:295, 1921.
12. Morpurgo, B.: Verhandl. d. deutsch. path. Gesellsch. **14**:259, 1910.
13. Hill, R. T.: J. Exper. Zoöl. **63**:203, 1932.
14. Friedberger, E., and Nasetti: Ztschr. f. Immunitätsforsch. u. exper. Therap. **2**:509, 1909.
15. Ranzi, E., and Ehrlich, H.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **3**:38, 1909.
16. Steiner, G., and Steinfeld, J.: Klin. Wchnschr. **6**:1597, 1927.

Zacherl [17] showed that the leukocyte counts of the parabionts often differ conspicuously, and numerous observations of our own confirm this finding. The mechanism that maintains the numbers of leukocytes and erythrocytes in each parabiont is not overcome by the union, and it is not known to what extent vascular communications exist between parabiotic mice.

The passage of plasma between parabionts was investigated by injecting into one of the paired mice mouse or rabbit serum containing agglutinins against Bacillus typhosus and then titrating the agglutinins in the blood of both mice. The titers of the agglutinins in one pair four hours after the injection of the agglutinating serum are given in table 6.

The titers for a second pair were higher, but the ratios were similar.

Since immune globulins are among the largest substances in the serum, it can be assumed that hypothetic humoral substances inhibiting

TABLE 6.—*Passage of Plasma Between Parabionts As Shown by Transfer of Agglutinins*

	Titer of Agglutination at Given Dilution of Serum					
	1:2	1:6	1:18	1:54	1:162	1:486
Parabiont given injection	+	++	++	++	++	0
Parabiont not given injection	+	++	++	++	0	..
Normal mouse (control)	0	0	..	..	..	..

the growth of malignant tumors if present in the circulation of one mouse would pass freely to its parabiont.

The experiments described in which leukemic cells injected into resistant mice produced leukemia in their uninoculated susceptible parabionts indicate that white blood cells pass from one animal to the other.

Since the erythrocytes of a mouse are smaller than the leukocytes, it seemed probable that they pass from one parabiont to the other in larger numbers than do the leukocytes. The passage of erythrocytes from one parabiont to the other was demonstrated in two ways:

1. Rat erythrocytes injected intravenously into one parabiont were demonstrated in the blood of the other by agglutination tests. A typical experiment was as follows:

Eleven days after establishment of parabiosis 0.3 cc. of heparinized rat blood was injected intravenously into one parabiont, and thirty and sixty minutes later 0.5 cc. was injected. Agglutination tests were made twenty minutes, one hundred and twenty minutes, twenty-four hours and forty-eight hours after the first injection with the serum of mice that had received repeated injections of washed rat erythrocytes. The results are given in table 7.

17. Zacherl, H.: Strahlentherapie **23**:272, 1926.

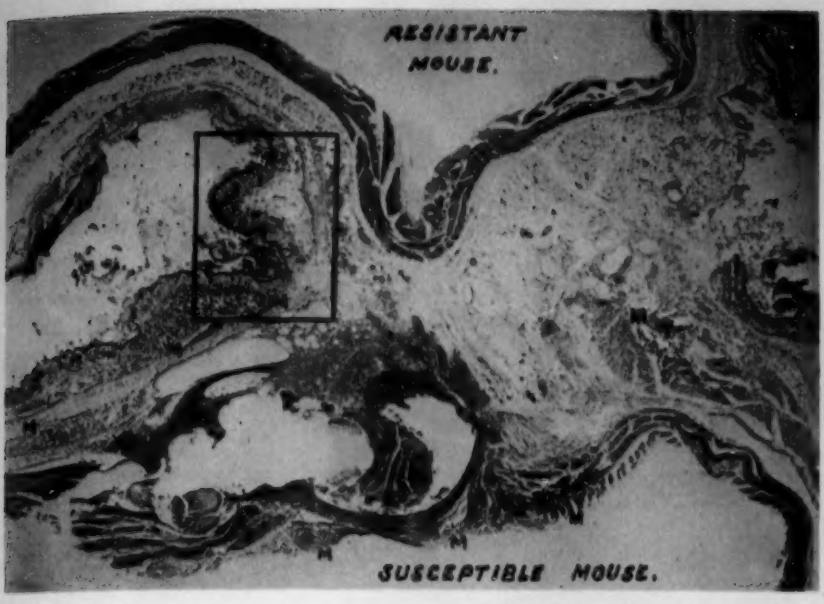

Fig. 1.—Site of anastomosis between a mouse with transmitted myeloid leukemia and a nonleukemic parabiont; hematoxylin and eosin; approximately × 15. *M* indicates groups of immature myeloid cells in the tissues of the susceptible mouse. Such cells are absent in the tissues of the resistant mouse.

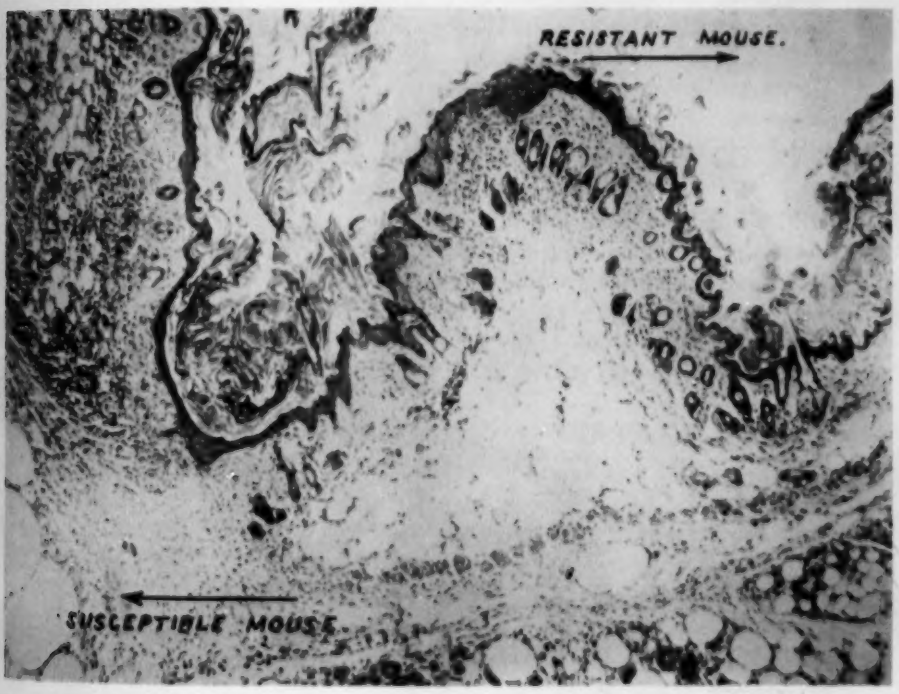

Fig. 2.—Higher magnification of the area indicated in figure 1; approximately × 80. There is extensive infiltration by leukemic cells in the subcutaneous tissue of the susceptible mouse, but no leukemic cells are present in the neighboring tissue of the resistant mouse.

This experiment shows that rat erythrocytes were present in small numbers in the blood of the nontreated parabiont twenty minutes after its twin had received the first injection and large numbers of rat erythrocytes were present one hundred and twenty minutes after this first injection.

2. When 0.3 cc. of heparinized chicken blood was injected repeatedly intravenously into one member of a parabiotic pair eleven days after union, the typical nucleated oval avian erythrocytes were found in the organs of the mouse which had received no injection.

Since erythrocytes are nonmotile, their presence in the mouse which had received no injection establishes the presence of blood vascular channels between parabiotic mice.

Microscopic examination of the sites of anastomosis failed to show any extravascular migration of leukemic cells from one animal to the

TABLE 7.—*Passage of Rat Erythrocytes Between Parabiontic Mice as Shown by Agglutination Tests*

	Titer of Agglutination in			
	Parabionts		Controls	
Time After First Injection	Mice Given Injection of Erythrocytes	Mice Not Given Injection	Rat Erythrocytes	Mouse Erythrocytes
20 minutes	+++	+	++++	0
120 minutes	+++	++	++++	0
24 hours	+++	+++	++++	0
48 hours	+++	+++	++++	0

other. Figures 1 and 2 show the site of anastomosis between a mouse with myeloid leukemia and a resistant mouse. There is perfect union of the skin. There is advanced leukemic infiltration in the subcutaneous tissue of the leukemic mouse close to the site of union, but the subcutaneous tissue of the resistant animal at a distance of a few hundred microns is free from infiltration. There are many capillaries and dilated lymphatics, as described by Rössle.[10]

COMMENT

When viable leukemic cells were inoculated into a susceptible mouse in parabiosis with another susceptible mouse, leukemia developed in both. Since it has been shown [18] that leukemia can be transmitted only by the introduction of viable leukemic cells, this observation indicates that the malignant cells passed from the inoculated to the uninoculated animal. The leukemic infiltrations of both the inoculated and the uninoculated mouse were about equally extensive, and both

18. Furth, J.: J. Exper. Med. **61**:423, 1935. Furth and others.[9]

died approximately the same number of days after inoculation as single susceptible control mice similarly inoculated. Hence parabiosis has no influence on the susceptibility to leukemia or on the length of life after inoculation.

When susceptible and resistant mice were paired and the former were inoculated with leukemic cells, leukemia developed in all but 1 of the susceptible and in none of the resistant mice. This shows that neither resistance nor susceptibility was conveyed from one parabiotic animal to the other. The life of the susceptible mouse was not prolonged by union with the resistant parabiont.

Inoculated resistant mice in parabiosis with susceptible mice failed to show leukemia, while in many of the susceptible uninoculated mice leukemia developed. This again indicates that neither susceptibility nor resistance was transferred from one parabiont to the other. In many instances viable malignant cells passed from the inoculated resistant to the uninoculated susceptible animal, as indicated by the development of leukemia in the latter. In other instances the leukemic cells were probably destroyed in the inoculated resistant mouse and viable leukemic cells failed to enter the susceptible animal.

Mice of a stock partially resistant to certain strains of leukemia did not acquire leukemia in a higher percentage of cases when inoculated with leukemic cells while joined in parabiosis with susceptible mice than when inoculated singly; nor was the course of the disease modified by this procedure. When partially resistant animals were joined to susceptible mice and the latter inoculated, leukemia developed in only a very small percentage of the partially resistant ones, although all the susceptible mice died of the disease. This indicates that the mice of the partially resistant stock in most cases were able to destroy the small numbers of leukemic cells that reached their circulation.

The present studies indicate that leukocytes pass from one animal to the other. The passage of erythrocytes is generally assumed, but the data in the literature do not indicate whether the transfer of substances from one parabiont to the other occurs by way of blood or by way of lymphatic channels. Microscopic sections show both capillaries and dilated lymphatics at the site of anastomosis. We have undertaken to demonstrate the passage of erythrocytes by blood vascular channels from one parabiont to the other. Rat erythrocytes are of approximately the same size as mouse erythrocytes, and their passage from one parabiont to the other can be demonstrated by agglutination with the serum of mice that have been immunized against rat erythrocytes. Subsequently we found that chicken erythrocytes can be demonstrated in the organs of one parabiont after injection of repeated doses into the other.

Since there is a communication of blood vessels between leukemic and resistant parabiotic mice, small amounts of blood may constantly pass from the resistant to the leukemic animal, and vice versa. Yet the duration of illness of mice inoculated with leukemic cells in parabiosis with mice resistant to leukemia was not prolonged as a result of parabiosis. Hence these experiments suggest that it is unlikely that the course of leukemia can be profoundly modified by continuous transfusion of normal blood.

These experiments demonstrate that resistance and susceptibility of mice to transmissible leukemia are not governed by humoral factors.

Our studies concern only inherited resistance to leukemia. It is possible that acquired resistance to neoplasms is associated with circulating antibodies.

SUMMARY

Parabiosis has been used as a means of studying the factors of resistance and susceptibility to transmissible leukemia in mice.

Leukemic cells pass from one susceptible parabiont to the other and produce leukemia in both.

The development of leukemia in a susceptible mouse inoculated with leukemic cells is not influenced by its parabiotic union with a resistant mouse.

Leukemic cells injected intravenously into a resistant mouse may pass to a susceptible parabiont, producing leukemia in the latter without ill effect on the former.

Resistance to transmissible leukemia in mice of a stock only partially resistant to leukemia remains likewise unaltered by parabiosis with susceptible mice.

The presence of blood vascular anastomosis between parabionts has been demonstrated by the passage of erythrocytes from one parabiont to the other.

CONCLUSIONS

Inherited resistance and susceptibility to leukemia of mice are not transmitted from one parabiont to the other; hence it is unlikely that they are governed by humoral factors.

The passage of erythrocytes from one parabiont to the other indicates the presence of blood vascular anastomosis between parabiotic mice.

REACTION OF MOUSE SKIN TO VARIOUS REDUCED AND PARTIALLY OXIDIZED SULFUR COMPOUNDS

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Mouse skin can be used to achieve that highly desirable result in studies of growth, the separation of various phases of growth and development from each other. Thus proliferation can be separated from cell differentiation, and this, in turn, from cell and tissue organization. Cells can be counted and measured; volumes of epiderm can be recorded, and so on. At least, this can be done anatomically; reasonable physiologic deductions also may be drawn, though at this and all other times it must be emphasized that, especially in incompletely differentiated cells, it is impossible to deduce physiologic states and possibilities from appearances. If the whole of growth and development is epigenetic and not preformed, so also is the development of just those kinds of cells which are most important in studies of both normal and abnormal growth, viz., incompletely differentiated cells. Their differentiations are developed, not merely unfolded, from manifold internal potencies determined and allowed to reach quantitative degrees by their envirnoment.[1]

This separation of growth processes and these physiologic considerations are made seldom indeed, and this omission has led to utter confusion in meanings, a woeful lack of understanding among various workers and an impossibility of comparing results. We cannot emphasize too strongly that this lack of attention has caused and is causing a degree of confusion that is most regrettable.

We define cell differentiation as that series of propensities which cause a cell to rearrange its chemical makeup so that it becomes specific from nonspecific, loses its multiple competence or potency, gradually loses its power to multiply and takes its place anatomically and physiologically as part of a functioning tissue in an organism. Organization we define here as that series of processes by which cells of various differenti-

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This investigation was aided by a grant from the Blanche and Frank Wolf Foundation.

1. Weiss, P.: Principles of Development, New York, Henry Holt & Co., 1939.

ations gather in integrated groups to form organs and parts which will and do function in an organism. Our views as to how malignant tendencies fit into this picture of growth and development have been expressed before.[2]

EXPERIMENTAL PROCEDURE

White petrolatum was used as the vehicle in which were incorporated 0.5 per cent thiocresol, cystine-disulfoxide, methionine sulfoxide, dl-methionine, cystine and cysteine sulfinic acid. Dibenzanthracene in 0.3 per cent mixture was also used as a sort of pathologic control. Each mixture was rubbed into the skin of the right ears and backs of 10 mice daily for three weeks. At the end of three weeks (eighteen applications) the ears and slices of skin were removed, fixed in Susa's solution, embedded in paraffin, cut at 7 microns and stained with hematoxylin and eosin. The images of the sections as projected from an Edinger apparatus were traced on paper, the lengths measured, and the area measured with a planimeter. The square millimeter area occupied under the aforementioned conditions by the skin was computed for 100 mm. lengths. The standard deviations obtained from the raw data collected from the individual sections are used in computing the ratios. The compounds used were prepared and purified by Toennies and Lavine.[3]

	Thickness of Skin, Ratios
Controls (white petrolatum)	1
Thiocresol	3
Cystine disulfoxide	1.2
dl-methionine	1
Methionine sulfoxide	1
Cysteine sulfinic acid	1.1
Cystine	1
Dibenzanthracene	1.5

COMMENT

Thus far, no sulfur compound has been found which diminished the thickness of mouse skin, i. e., inhibited normal replacement proliferation. We draw no conclusions from this except that the experiments have not shown that the partially oxidized sulfur compounds used do not inhibit the rate of cell division of the skin, for we do not know at what rate desquamation occurred, and data on this are essential for evaluation of inhibition effects in this material. On the other hand, it has been shown that partially oxidized sulfur compounds do inhibit proliferation in special cases. Thus cystine disulfoxide inhibits the

2. Reimann, S. P.: Biology of the Cancer Cell, in Symposium on Cancer: Addresses Given at an Institute on Cancer Conducted by the Medical School of the University of Wisconsin, Madison, Wis., University of Wisconsin Press, 1938, p. 114.

3. Toennies, G., and Lavine, T. F.: J. Biol. Chem. **113**:571, 1936. Lavine, T. F.: ibid. **113**:583, 1936. Toennies, G., and Kolb, J. J.: ibid. **128**:399, 1939.

proliferative phase in the growth of Obelia.[4] It inhibits the rate of growth of spontaneous mammary tumors,[5] a result duplicated by English workers with partially oxidized sulfur groups in sulfanilamide types of compounds.[6] Indeed, in connection with Hammett's[7] views on the physiologic activity of sulfur groups, it seems worthy of attention that the whole field of sulfanilamide therapy deals with suboxidized sulfur and with inhibition of proliferation.[8]

Qualitatively there was no difference from the normal in the appearance of the skins rubbed with the partially oxidized compounds.

In contrast, the sulfhydryl compound (thiocresol) had the usual effect of increasing the rate of cell proliferation, and thus there were produced more cells per unit of time per given area. A description of the qualitative changes has been reported[9] and so will not be repeated, but several pertinent conclusions will be mentioned that were strenghtened by these further experiments. These are:

1. The rate of cell multiplication can be speeded by application of a normal stimulus, viz., — SH..

2. With this stimulation the cells proceed to higher degrees of differentiation and organization.

3. Thus the potency or competence for differentiation and organization of cutaneous cells is greater than the normal realization of these processes.

4. Multiplication in normal cells, in cells treated with sulfhydryl and cells treated with partially oxidized sulfur compounds occurs only in the basal layer.

Fitted into the general knowledge of growth and development, the following remarks are pertinent. There is direct and proportional antagonism between differentiation and multiplication.[1] Tumors must arise from incompletely differentiated cells, therefore mostly from reserve cells or "spare parts." (The question of whether dedifferentiation of individual cells can occur will not be discussed here, though it is most pertinent.[1]) An increase in the rate of cell multiplication alone does not lead to malignant growth.[9] Cancer is not a disease of cell multiplication but one partly of cell differentiation and wholly of cell and tissue organization.[2] Finally, we have been dissatisfied for some years with the classification which places benign and malignant

4. Lavine, T. F.: Am. J. Cancer **25**:809, 1935.
5. Reimann, S. P., and Hammett, F. S.: Am. J. Cancer **26**:554, 1936.
6. Boyland, E.: Biochem. J. **32**:1207, 1938.
7. Hammett, F. S.: Protoplasm **11**:382, 1930.
8. Reimann, H. A.: Arch. Int. Med. **64**:362, 1939.
9. Hammett, F. S.: Protoplasm **13**:331, 1931. Reimann, S. P.: Am. J. Cancer **15**:2149, 1931; Arch. Path. **17**:764, 1934.

growths under one heading, viz., tumors; and the increasing number of "causes" being found for growths which have been classified for years as "benign tumors" crystallizes this sentiment when the causes are regarded in the light of the whole field of growth and development, including, as it does, embryonic and fetal growth and development, replacement, repair, physiologic hyperplasia and the other processes. We prefer calling benign tumors "growth anomalies," and we correlate them directly with such accepted anomalies of growth as the horseshoe kidney and the accessory spleen. Such anomalies are produced during embryonal times—but does growth stop when this period is concluded? Furthermore the old, purely anatomic factors cited in explanation of growth anomalies have been supplemented in many cases by physiologic ones in the form of organizers, gradients, fields and the like, to much benefit in experiment and correlation. Certainly the ordinary periductal fibroma of the breast has a a physiologic cause so definitely known that its "cause" can be predicated on its mere presence in the breast under certain conditions.[10] And the mechanical causes are there, too, just as they are assumed to have been present in the older, less complete explanations of growth anomalies. At all events, benign growths have an organization, while malignant ones do not, and cellular differentiation in benign growths is quite different anatomically (and physiologically by presumption, for many reasons) from that in malignant growths. Therefore we give a definition of malignancy which was timidly advanced several years ago but which we now repeat with more assurance: "A malignancy is a mass of cells which arises from and continues to proliferate within an organism as a result of and in direct proportion to their degree of internal qualitative differences from the other cells of the organism with respect to the potencies of differentiation and organization particularly."

For an analysis of the dibenzanthracene-treated mouse skins from the present point of view see the previous report.[11]

Turning to the chemical aspects for leads and further explanations, we are confronted with many isolated facts. Probably the sulfur groupings do not stay as such when they are applied to cells, for the latter have broad capacities for bending the sulfur groupings into the locally required conditions, oxidizing or reducing them or establishing equilibriums within wide limits according to internal and environmental conditions.

Cystine disulfoxide supported growth in weight in feeding experiments.[12] It also caused thickening of mouse skins a triflle through

10. Reimann, S. P.: An Ovarian Tumor Diagnosed from a Breast Tumor, in Libro de oro dedicado al Prof. Dr. Angel H. Roffo en ocasion de sus bodas de plata con la cancerologia, 1910-1935, Buenos Aires, 1935.

11. Reimann, S. P., and Chatalbash, N.: Growth **1**:247, 1937.

12. Bennett, M. A.: Biochem. J. **33**:885, 1939.

an increase in the number of cells. Presumably this is because it liberates sulfhydryl, for in the test tube cystine disulfoxide decomposes rather slowly into sulfhydryl and sulfinic acid.[3]

$$2(RSO)_2 + 2H_2O \rightarrow 2RSOH + 2RSO_2H$$
$$\downarrow$$
$$RSH + RSO_2H \text{ .}$$

On the other hand, cystine disulfoxide inhibited the rate of growth of spontaneous mammary tumors in mice [5] and also the proliferative phase of the growth of Obelia.[4] Again, in the test tube cystine disulfoxide reacts instantaneously with sulfhydryl groups,[3] thus applying brakes to proliferation.

$$(RSO)_2 + RSH \rightarrow RSSR + RSO_2H$$

Of the two aforementioned possibilities, from the mass law it seems that in the case of dearth of sulfhydryl groups the first reaction would be favored; in the presence of sulfhydryl the second reaction would occur.

Methionine may be converted to cystine and cysteine in the mammalian body.[13] It supports growth in weight and stimulates proliferation in Obelia but not in mouse skin. For this reason we conclude at present that the epiderm does not convert methionine to cysteine, for these experiments have shown that cystine does not increase skin proliferation, whereas cysteine does.[14]

Neither cysteine sulfinic acid nor methionine sulfoxide appears to inhibit tumor growth; [15] they cause no change in mouse skins; cysteine sulfinic acid does not support growth in weight, but methionine sulfoxide does.[16] Both compounds react very slowly with SH in the test tube.[3]

$$RSO_2H + 3RSH \rightarrow 2RSSR + 2H_2O$$
$$\text{Meth. SO} + 2RSH \rightarrow \text{Meth. S.} + RSSR + H_2O$$

Herein may be an explanation of the failure of proliferation-inhibiting effects.

Neither cysteine sulfinic acid nor methionine sulfoxide yields sulfhydryl on decomposition. But the growth in weight evidence seems to indicate that methionine sulfoxide is readily shunted by the body into the normal pathways of methionine metabolism, whereas the body does not seem capable of utilizing the sulfinic acid similarly—this in spite

13. Toennies, G.: Growth **1**:337, 1937.

14. Brunsting, L. A., and Simonsen, D. G.: J. A. M. A. **101**:1937, 1933.

15. Preliminary experiments, to be pursued further, have demonstrated this.

16. Bennett, M. A.: Biochem. J., to be published.

of the fact that in vitro the reduction of the latter to cystine requires less energy than the reduction of methionine sulfoxide to methionine.

Unfortunately cystine disulfoxide, which is highly reactive with —SH, is also highly active in undergoing spontaneous decomposition, which leads to the inert compounds cystine and sulfinic acid.[3] The technical question arises, therefore, as to whether it is possible to discover or design a sulfoxide which will have high reactivity for sulfhydryl, and therefore proliferation-inhibiting properties, with, at the same time, a sufficiently increased resistance to spontaneous decomposition to permit it to have more than transitory existence in the body.

SUMMARY

Mouse skins have been rubbed with thiocresol, cysteine sulfinic acid, cystine, cystine disulfoxide, dl-methionine, methionine sulfoxide and dibenzanthracene. The sulfhydryl compound increased the rates of cell proliferation and the cells subsequently differentiated and organized to higher degrees than in normal skin. The other compounds showed no such effects. Some of the chemical relationships of these compounds are discussed in the light of their biologic behavior on mouse skin as well as in feeding and other experiments.

On correlating these findings with the general knowledge of growth and development, it is again concluded that malignancy begins in incompletely differentiated cells or "spare parts," that the changes of malignancy are qualitative changes in cell potencies and on the basis of these considerations and others a definition of a malignant growth is again proposed as follows: A malignant growth is a mass of cells which arise from and continue to proliferate within an organism as a result of and in direct proportion to their degree of internal qualitative difference from the other cells of the organism with respect particularly to the potencies of differentiation and organization.

EFFECT OF DL-METHIONINE AND L-CYSTEINE ON THE CLEAVAGE RATE OF MAMMALIAN EGGS

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Sulfhydryl has been shown to play a dominant role in cell division in about fifty species of animals and plants and also in many different processes in which cell division takes place. The experiments reported here were designed to show directly that sulfhydryl has the same effect of increasing rates of cell division in fertilized mammalian ova, the rabbit ovum being chosen as a test object. Other evidence that sulfhydryl performs this function in ova is that from the experiments of Pincus,[1] Gregory and Castle [2] and Gregory and Goss.[3] The last authors showed that there is a greater concentration of sulfhydryl groups as glutathione in the developing fetuses of giant strain rabbits and chickens than in the corresponding smaller size strains.

The use of sulfhydryl in these experiments requires exceptional chemical care, since this group is extremely labile, as one would expect it to be if it plays a dominant role in such a dynamic process as cell division. The technic and precautions have been outlined in extenso by Hammett,[4] particularly in connection with the analysis of the negative results obtained by some workers.

It is again emphasized that sulfhydryl affects only the proliferative phase of cell activity. What cells do further in the way of differentiation and organization are functions of other mechanisms (Hammett [5]). Obviously the early development of the fertilized rabbit ovum is mostly concerned with growth by proliferation. Differentiation in the ovum does not become evident until the middle of the blastocyst stage.

From the Lankenau Hospital Research Institute.

This investigation was aided by a grant from Mrs. L. Elizabeth Nax.

1. Pincus, G.: The Metabolism of Ovarian Hormones Especially in Relation to the Growth of the Fertilized Ovum, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 44.

2. Gregory, P. W., and Castle, W. E.: J. Exper. Zoöl. **59**:199, 1931.

3. Gregory, P. W., and Gross, H.: J. Exper. Zoöl. **66**:155, 1933.

4. Hammett, F. S.: Protoplasma **22**:489, 1934.

5. Hammett, F. S.: Nature, London **141**:82, 1938.

It was first shown by Lewis and Gregory [6] and later by Pincus [7] that fertilized rabbit ova grown under ordinary conditions of tissue culture will cleave regularly up to the initial stages of blastocyst expansion and that they will collapse if cultured beyond this point. Ova grown in Carrel flasks or in the circulating cultures described by Pincus and Werthessen [8] will continue to expand beyond this point.

The compounds used in this study were dl-methionine, 99.7 per cent pure by analysis, prepared by C. S. Marvel at the University of Illinois, and l-cysteine, 98 per cent pure by analysis, prepared by G. Toennies of this institute.

APPARATUS AND METHOD

Unicellular fertilized rabbit ova and ova in the morula of development were obtained by the usual flushing-out technic. Blood was first obtained from the doe by puncture of the heart and then stored overnight in the refrigerator. The serum was then obtained by centrifugation and stored in sterile tubes until the following day. In this manner it was possible to keep on hand sufficient fresh serum, approximately a day old. Serum older than two days was never used, although Pincus and Werthessen [8] claimed that serum as old as eleven days will still support the growth of the ovum. Bouillon cultures were always prepared for each batch of serum to insure against contamination.

Immediately after the cardiac puncture the animal was put to death, and the viscera were exposed under sterile conditions and the tubes and uteri excised. It is a wise precaution to insure against dust contamination from the air in the room by first spraying with 0.1 per cent phenol solution. The medium used for flushing consisted of equal parts of Tyrode's solution, which had previously been sterilized by filtration, and rabbit blood serum obtained from the doe killed the day before.

For ova younger than thirty hours after copulation flushing was usually done from the proximal portion of the tube with a sterile syringe. Ova in more advanced stages of development were best obtained by flushing from the fimbriated end of the tube. Washings were recovered in sterile watch crystals.

Within a few minutes the ova settle to the deepest portion of the watch crystal and may be easily located at a magnification of about 50. The ova are immediately transferred to the culturing apparatus by means of sterile pipets. Care must be exercised in this final step in order that damage to the ova may be avoided.

Two types of culturing devices were used. Watch crystals containing 1 or 2 cc. of serum were placed in sterile moist chambers consisting of sterile Petri plate and a layer of moist filter paper placed in the bottom of the dish. Small and large Carrel flasks containing varying amounts of blood serum were also employed. These were used when growing blastocysts were to be observed.

Three series of experiments were conducted in order that accurate checks on each might be obtained. Unicellular ova were first cultured and the number of blastomeres recorded at definite intervals of time. By keeping the time constant one is able to observe the acceleration of cellular activity produced by sulfhydryl in relation to the rate of mitosis in control ova. Ova in the ten to twelve cell stage were next cultured and the morphologic characteristics of each ovum observed at

6. Lewis, W. H., and Gregory, P. W.: Science **69**:226, 1929.

7. Pincus, G.: The Eggs of Mammals, New York, The Macmillan Company, 1936.

8. Pincus, G., and Werthessen, N. T.: J. Exper. Zoöl. **78**:1, 1938.

the termination of the culture period. This method of observation was found to be more advantageous because it was less difficult to tell when an ovum was in the morula or blastula, than to count the number of cells beyond a certain point.

Other ova were studied in tissue culture by observing increase in diameter and morphologic changes over a longer period. Such ova were isolated in the eight to ten cell stage and cultured in small and large Carrel flasks. It was found that before the advent of blastocyst expansion the diameter of the enclosed mass of blastomeres increased more rapidly than the mean diameter of the ovum as measured from one surface of the zona pellucida to the other. It is obvious that the zona pellucida plays a scant part in growth and that it is the mass of blastomeres which is actually concerned with growth. It was therefore felt that measurements of the diameter of the cell mass rather than the diameter of the entire ovum would give a more accurate picture in relation to the growth of the fertilized ovum.

TABLE 1.—*Stimulation of Cellular Activity in Ova Produced by dl-Methionine After Five Hours of Culturing*

Animal	Ova	Cells in Each	Medium	Cells at Conclusion of Experiment
R8A	6	1	Control of serum from animal R 7 A	2
		2		2*
		2		2*
		2	1 cc. of serum containing 0.6 mg. dl-methionine	3*
		2		5
		2		5

* This number indicates cellular activity.

RESULTS

In the first series of experiments six fertilized ova were recovered from doe R8A approximately twenty-six hours after copulation, five in the two cell stage and one as yet undivided. Three ova were placed in a watch crystal culture chamber containing 1 cc. of blood serum from rabbit R7A as a control. The remaining ova were placed in another watch crystal chamber containing 1 cc. of the same serum, to which had been added 0.6 mg. of dl-methionine.

At the conclusion of a five hour period these ova were observed. The one control ovum which was unicellular at the beginning of the experiment had divided. The remaining two ova were still in the two cell stage, although both when studied closely showed evidences of dividing again.

Ova cultured in methionine were definitely stimulated. One ovum showed three distinct blastomeres and evidence of dividing again, while the remaining two ova contained five blastomeres each. The results of this experiment are shown in table 1.

Five unicellular fertilized ova were recovered from rabbit R9A approximately twenty-three hours after copulation. Two of these ova

were placed in a watch crystal culture chamber containing 1 cc. of serum obtained from rabbit R8A as a control. The remaining three were placed in a watch crystal culture chamber containing 1 cc. of the same blood serum, to which had been added 0.6 mg. of dl-methionine. The results of twenty-two hours of culturing are given in table 2. Of the two ova placed in control culture, one continued to the four cell stage and the other to the two cell stage. Ova cultured in the presence of 0.6 mg. dl-methionine in 1 cc. of serum definitely showed acceleration of cellular activity and had already reached the six and eight cell stage with the exception of one which did not divide.

In the second series of experiments, ova in the eight to ten cell stage were recovered and cultured for twenty-two hours in watch crystal culture chambers. Observation has shown that ova older than thirty hours after copulation are less susceptible to "shock" than younger ova. At the

FIG. 2.—*Stimulation of Cellular Activity in Ova Produced by dl-Methionine After Twenty-Two Hours of Culturing*

Animal	Ova	Cells in Each	Medium	Cells at Conclusion of Experiment
R9A	5	1	Control of serum from animal R 8 A	4
		1		2
		1	1 cc. of serum containing 0.6 mg. dl-methionine	1
		1		6
		1*		8

* A cleavage furrow was evident at the time of culture.

conclusion of the experiment these ova were studied in order to determine what stage of development had been attained.

Seven ova in the eight to ten cell stage were recovered from doe R10A approximately fifty hours after copulation. Two ova were placed as a control in a watch crystal culture chamber which contained 1 cc. of serum from doe NR9A. One ovum was placed in a hanging drop culture. Of the remaining ova, two were placed in an identical culture chamber containing the same amount of blood serum, to which had been added 0.26 mg. of l-cysteine, and two in another culture chamber containing 0.52 mg. of l-cysteine.

After twenty-two hours of culturing the two ova placed in a watch crystal culture were in the early morula of development (fig. 1 *A* and *E*). The blastomeres were comparatively large and easily discernible, and as yet there were few signs of any peripheral flattening. The one ovum placed in a hanging drop was in the very early morula. The blastomeres were also large, and few showed any signs of peripheral flattening.

The two ova cultured in the presence of 0.26 mg. of l-cysteine were in the blastocyst when finally observed (fig. 1 *A*). The blastomeres showed signs of differentiation. They were smaller than those of the control ova and far greater in number. The expanding vesicle and cleft

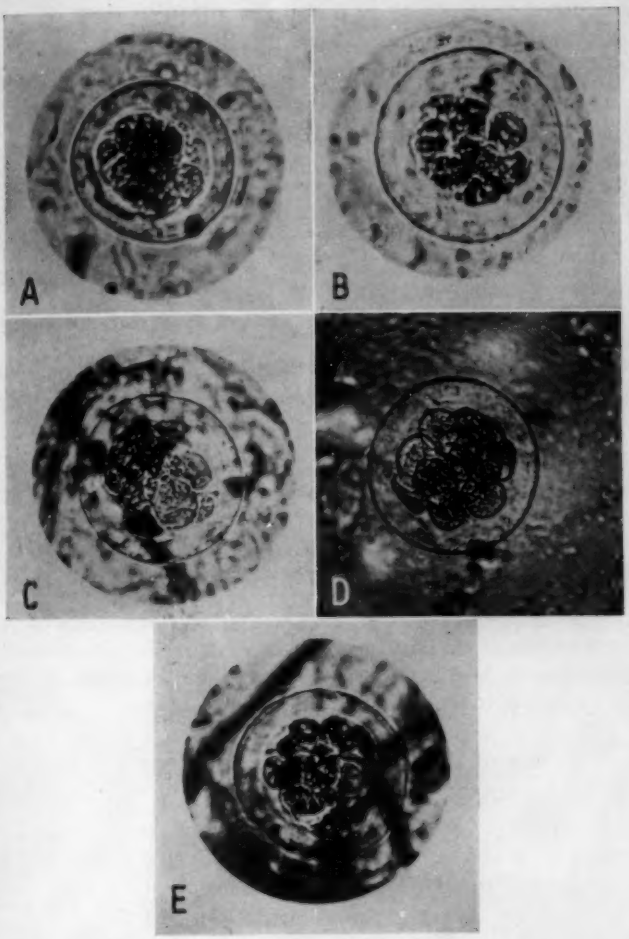

Fig. 1.—*A*, final appearance of ovum cultured in 1 cc. of serum containing 0.26 mg. of l-cysteine. *B*, final appearance of ovum cultured in 1 cc. of serum containing 0.52 mg. of l-cysteine. *C*, final appearance of second ovum cultured in 1 cc. of serum containing 0.52 mg. of l-cysteine. *D*, control ovum in hanging drop. *E*, control ovum in 1 cc. of serum.

demarcating it were easily seen. As yet little expansion of the blastocyst cavity had occurred.

The remaining two ova, grown in culture containing 0.52 mg. of l-cysteine, likewise showed stimulation of cellular activity (fig. 1 *B* and

C). The blastomere of these ova were likewise smaller and more numerous than those of the control ova. However, these were not quite as far developed as those cultured in a medium containing 0.26 mg. of l-cysteine.

The cultures were continued, and by the following day the control ova, excluding the one ovum in a hanging drop, had ceased developing beyond the very early morula. The ova cultured in cysteine had already collapsed, which indicated that these blastocysts had been expanding.

A third series of experiments were performed in which eight to ten cell ova were cultured in Carrel flasks for sixty-four hours. In this series increase in diameter of the enclosed cluster of blastomeres was noted and growth curves constructed from the data (fig. 2). Two control ova from rabbit R10A were placed in small Carrel flasks containing 1 cc. of rabbit serum. These ova never progressed beyond the morula. They did, however, show uniform growth up to this point. This is attributable to the fact that at this point the blastomeres were pressing against

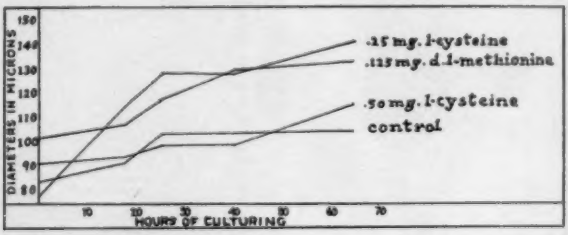

Fig. 2.—Curves of the proliferative growth of the blastomeres of rabbit ova when cultured in 1 cc. of the serum of a doe to which various amounts of l-cysteine or dl-methionine had been added, as shown by the diameters of clusters of blastomeres at various intervals during the period of culture.

the internal surface of the zona pellucida, which really begins expanding only after the blastocyst begins to expand. As is seen in figure 2, the curve for the control ova is lowest.

Ova cultured in the same amount of blood serum containing 0.25 mg. of cysteine showed rapid proliferation of blastomeres with consequent increase in diameter. This was most marked during the first twenty-five hours of culturing (fig. 2). The average diameter of the enclosed cell mass remained unchanged during the following fifteen hours of culturing, after which another period of expansion began which lasted until the conclusion of the experiment.

When a concentration of 0.50 mg. of cysteine was employed, there was little if any stimulation of blastomere proliferation. There was a slight increase in diameter during the first eighteen hours of culture. This was followed by a more rapid increase in diameter during the following seven hours. During the following fifteen hours the rate of increase

in diameter was considerably reduced. At the conclusion of the experiment these ova had obtained a peak far from that of ova cultured in a concentration of 0.25 mg. of cysteine.

Ova cultured in the presence of 0.125 mg. of dl-methionine showed a very definite increase in diameter during the entire time of culturing, terminating at a point lower that that observed with a concentration of 0.25 mg. of l-cysteine.

COMMENT

Pincus [1] showed that glutathione, the tripeptide of cysteine, glycine and glutamic acid, added to circulating cultures of growing rabbit blastocysts, definitely causes an increase in blastocyst size. There is also a simultaneous decrease in the endometrial glutathione during the preimplantation period of ovum growth, which is obviously attributable to the rapid utilization of glutathione by the growing ova and endometrium. Hammett,[9] in offering a possible explanation of the function of this naturally occurring molecule, has postulated that "nature has developed in one and the same chemical compound a complex which conditions if it does not determine the course of the several basic and essential processes concerned in developmental growth." The roles of the three amino acids of which glutathione is the derivation have already been adequately investigated by Hammett and reported in a long series of papers. According to this, then, the free sulfhydryl group of cysteine definitely stimulates the proliferative phase of growth, while glycine and glutamic acid are concerned with other phases of developmental growth which follow proliferation.

It seems possible, then, that in the growing ovum, as in other proliferating cells, sulfhydryl, the natural stimulant of growth, is made available to these dividing cells by utilization of naturally occurring glutathione. An analysis of figure 2 shows that a concentration of 0.25 mg. of cysteine was most effective in producing an almost instantaneous increase in the rate of cellular activity in the proliferation phase of developmental growth of the fertilized rabbit ovum, while a concentration of 0.5 mg. of cysteine was far less effective.

Methionine, like cysteine, definitely stimulates the proliferative phase of growth in Obelia geniculata (Hammett [4]), leaving the developmental phases of differentiation and organization unaffected. This same effect has been observed with growing rabbit ova (fig. 2).

These ideas are further substantiated by the fact that methionine and cysteine promote normal mammalian growth. Bennett [10] demonstrated that l-cystine could be replaced by methionine and various other sulfur-containing amino acids in the diet of rats.

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9. Hammett, F. S.: Science **79**:457, 1934.
10. Bennett, M. A.: Biochem. J. **33**:885, 1939.

The possible chemical relationship between methionine and cystine in metabolism was first pointed out by Rose [11] when he showed that the role of cystine in growth is secondary to methionine.

Hammett [12] showed that sulfhydryl is the essential stimulant to cell division. From this it seems evident that in the case of either an absence or a deficiency of sulfhydryl, methionine may serve as a source of free sulfhydryl groups. It is now definitely known that methionine can go to cystine or to cysteine and that this reaction is not reversible. This was directly proved when Tarver and Schmidt [13] demonstrated that radioactive sulfur (S^{35}) when fed to rats as methionine may appear as cystine.

SUMMARY

Ova cultured in watch crystal cultures will collapse if cultured beyond the initial stages of the bastula. Expansion of the blastocyst can be observed when ova are cultured in Carrel flasks containing 2 to 3 cc. of rabbit serum.

Unicellular rabbit ova, isolated before twenty hours after copulation, usually will not grow in tissue culture.

The diameter of the enclosed mass of blastomeres increases more rapidly in diameter than does the entire ovum as measured from one surface of the zona pellucida to the other. Ova cultured in 1 cc. of blood serum containing 0.6 mg. of dl-methionine show a more rapid increase in the number of blastomeres over a period of five hours than do ova grown as controls. Ova in two cell condition are likewise stimulated to rapid blastomere proliferation.

Ova cultured in 1 cc. of blood serum containing 0.26 mg. of l-cysteine show an immediate and rapid increase in blastomere proliferation, while ova cultured in the same amount of blood serum containing 0.52 mg. of l-cysteine are not nearly so effectively stimulated. Ova cultured in Carrel flasks show a rapid increase in diameter when 0.25 mg. of l-cysteine is added to each cubic centimeter of fresh rabbit blood serum. Ova cultured under the same conditions in the presence of 0.50 mg. of l-cysteine are not so effectively stimulated to increase in diameter. Ova grown under the same conditions in the presence of 0.125 mg. of dl-methionine were also stimulated and showed a definite increase in diameter.

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MORPHOLOGIC APPEARANCES OF SPIROCHETAL REPRODUCTION IN TISSUES

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DETROIT

Certain morphologic appearances of spirochetes will be described that are interpreted as phenomena connected with the reproduction of these organisms in living tissues. In addition, evidence will be presented which indicates that there is a particular tissue reaction that depends on a characteristic phenomenon of spirochetal reproduction.

Spirochetal reproduction in the tissues can be studied best in (1) the tissues of patients in the very early stages of a spirochetal disease, during which time a large number of spirochetes are present—e. g., the tissues of patients with congenital syphilis and the tissues of patients with untreated acute dementia paralytica—and (2) the organs of animal carriers chronically infected with spirochetes, e. g., rats with leptospiral infection.

In several spirochetal diseases, the stage immediately before the crisis is characterized by the presence in the blood stream of densely clustered masses of spirochetes. These are called agglomerations. In mice and rats infected with the organism of relapsing fever these agglomerations appearing in the blood stream mark the end of a relapse (fig. 1 *A*). Again, just prior to the termination of the disease similar agglomerations are seen in chickens infected with Spirochaeta (or Borrelia) gallinarum. In both of these diseases (relapsing fever and spirochetosis gallinarum) high serologic immunity develops; this immunity is probably related to the phenomenon of agglomeration.

As can be seen in dark field preparations and also in silver mirror preparations of blood smears [1] in the very early stages of experimental relapsing fever, the spirochetes divide by transverse fission. The spirochetes of relapsing fever and of spirochetosis gallinarum are prone to reproduce in the blood stream, and only in very late stages of these diseases do they invade parenchymal parts. When they are no longer able to live in the blood stream, they may still exist in the parenchyma of the central nervous system. The period of their persistence in the brain is very short in spirochetosis gallinarum. However, in experi-

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1. Steiner, G.: J. Lab. & Clin. Med. **23**:293, 1937.

mental relapsing fever in mice, as has been shown by Buschke and Kroo,[2] Jahnel,[3] myself [4] and others, the spirochetes may persist in the brain as single individuals for a long period despite a high content of antibodies in the serum. During this time, which lasts for a year or more, the mice are immune to repeated inoculations. The same effect is seen in cases of dementia paralytica in which the treatment has included inoculation with the spirochetes of relapsing fever (Borrelia duttoni); borrelias are found in the brain for a rather long time after inoculation, although they are no longer present in the blood stream.[5] There seems to be no doubt that the brain affords some kind of protection against the effects of immune bodies of the serum during a time when the organisms cannot live or multiply in the blood stream. These residual spirochetes of relapsing fever as long as they remain in the brain have lost their power of reproduction. However, if emulsions of brain containing the spirochetes are inoculated into normal mice, reproduction will again take place. So, although the immunologic properties may be sufficient to prevent reproduction of spirochetes in the brain, these protective properties do not suffice to kill all of the micro-organisms.

It is not my intention to deal here with the factors responsible for the peculiar protection of spirochetes in the tissues of the central nervous system against the killing effects of immunologic substances in the serum.[6] In this paper interest is centered about the morphologic appearances in the tissues which result when the local reproduction of spirochetes is initiated.

My material consisted of: (1) 3 cases of congenital syphilis in which tremendous numbers of spirochetes were seen in many organs of the body; (2) 58 cases of dementia paralytica, among which were 3 examples of early acute untreated stages of this disease, and (3) the kidneys and other organs of 10 rats naturally infected with Leptospira icterohaemorrhagiae. The organs were embedded in paraffin and stained for spirochetes by the gum mastic silver method.[7] Liquid cultures of spirochetes were made solid by use of gentle heat and then cut as solid blocks in frozen sections. When possible, smears and dark field examinations were made.

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6. Steiner, G., and Steinfeld, J.: Klin. Wchnschr. **4**:1995, 1925; **5**:650, 1926.

7. Steiner, G.: J. Lab. & Clin. Med. **25**:204, 1939.

In the cases of congenital syphilis the organs (liver, kidney, adrenal, lung and other organs) revealed immense numbers of spirochetes. Despite their numbers, the method of division of the individual spirochete could not be made out in the fixed tissues. It is certain, however, that the huge number of spirochetes seen in the organs must

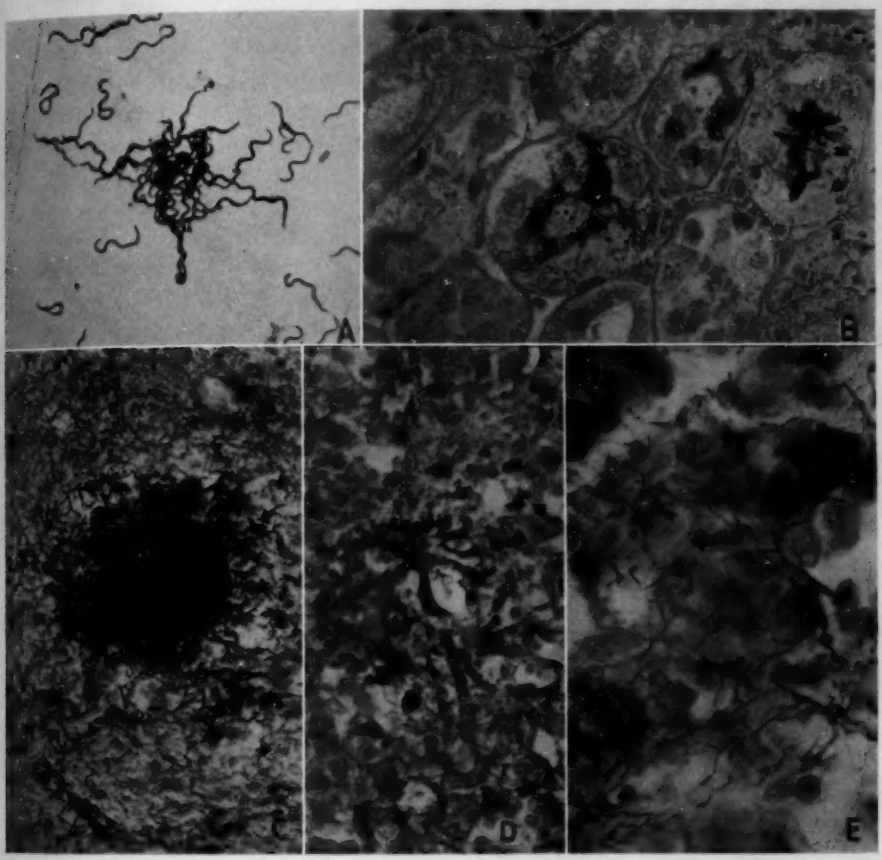

Fig. 1.—*A*, blood smear of a mouse with relapsing fever (Borrelia duttoni), showing an agglomeration of spirochetes at the end of a relapse. Silver mirror method; × 1,000. *B*, kidney of a rat naturally infected with Leptospira icterohaemorrhagiae. The massive centers of leptospiras are seen limited to the tubules. Gum mastic silver method; × 350. *C*, cerebral cortex in a case of acute dementia paralytica, showing a reproductive center (conglomeration); gum mastic silver method; × 450. Note the spread of spirochetes from the center and the pericolonial liquefaction of tissue. *D*, spirochetal reproductive center in the anterior lobe of the pituitary in a case of congenital syphilis; gum mastic silver method; × 300. A yellow center is shown, and the peripheral zones consist of a black ring with degenerating spirochetes and granules of spirochetal debris. In the surrounding tissues numerous well preserved spirochetes are seen. *E*, higher magnification of a neighboring area, surrounding the reproductive center of *D*; × 675.

have been the result of rather rapid spirochetal reproduction. In the tissues the organisms are distributed in two ways: (1) they are diffusely scattered; (2) they are accumulated in dense ball-like masses. It is to the latter appearance that I wish to draw special attention. Morphologically these ball-like masses are round or oval accumulations, made up of spirochetes closely packed together. No space for tissue elements seem to be left. With silver stains a characteristic feature of these large spirochetal masses is seen; the central and inner parts of the masses have a light yellow or brownish color, while the outer zone is black. The outer zone shows the usual black silver mirror appearance of spirochetes. Under the low power of the microscope these peripheral spirochetal coils tend to form a stellate pattern. At the periphery the spirochetes are arranged in raylike strands, the axes of which radiate from the center of the ball. The rays of this formation are made up of well defined black spirochetes. The central part of the mass consists of a compact yellow or brownish material which, by using a high power of the microscope, is seen to be formed by very fine, lightly stained spirochetal threads that are infinitely tangled. Such spirochetal conglomerations were seen in the liver, adrenals, hypophysis, intestinal walls and other organs in these cases of congenital syphilis.

As early as 1907 Benda [8] discovered massive conglomerations of spirochetes in the organs in cases of congenital syphilis; he called them "centers." Later on such conglomerations were seen by numerous pathologists. In 1906 Sträussler [9] described peculiar multiple miliary areas of necrosis in the cerebral cortex in a case of dementia paralytica, and later Gruetter,[10] Hauptmann,[11] Herschmann [12] and Schob [13] demonstrated balls of spirochetes as the causes of these necroses. In my own 3 cases of early dementia paralytica conglomerations of spirochetes were also seen. In acute dementia paralytica the spirochetes were distributed in exactly the same way as has been described for congenital syphilis.

For the following reasons I believe that such conglomerations represent *centers of spirochetal reproduction:*

1. The conglomerations are seen only in recent stages of active syphilis, both in congenital syphilis and in early and acute stages of dementia paralytica. They are never found in chronic syphilis. In tertiary lesions, where spirochetes are scanty or absent, the conglomerations are never seen.

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11. Hauptmann, A.: Monatschr. f. Psychiat. u. Neurol. **45**:59, 1919.
12. Herschmann, H.: Ztschr. f. d. ges. Neurol. u. Psychiat. **55**:27, 1920.
13. Schob, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. **95**:588, 1925.

2. The finding of conglomerations is always combined with that of a diffuse distribution of very numerous spirochetes in the neighborhood of the massive centers. Thus a very active phase of reproduction is indicated. In congenital syphilis conglomerations are found in different organs in the same case. In dementia paralytica, if spirochetal conglomerations are seen, they are usually found in large numbers in the cortical or other gray areas, always together with innumerable diffusely distributed spirochetes. From these centers a diffuse penetration of spirochetes into the neighboring tissue takes place.

3. In colonies from cultures of spirochetes in artificial mediums it has been possible to demonstrate a striking resemblance to the appearance of the conglomeration centers in congenital syphilis and dementia paralytica. First, sterile calf serum was coagulated in a sterile tube. Then, with a needle, a puncture canal was made in the center of the coagulated cylinder of serum. Next, blood of a mouse inoculated with the spirochetes of relapsing fever was diluted to 1:100,000 with physiologic solution of sodium chloride, and 2 cc. of this dilution was added to the tube with the coagulated serum. The tube was then centrifuged for five minutes and after that incubated at 30 C. for three days. The contents of the tube were then fixed in 10 per cent solution of formaldehyde U. S. P. and cut like ordinary tissue blocks. In some of these sections, after staining with silver nitrate, one or two star-shaped colonies were apparent grossly. Microscopically, these colonies showed yellowish centers from which strands of shiny black spirochetes radiated peripherally (fig. 2). The central parts showed innumerable spirochetes having only a yellow color.

There can be no doubt that these spirochetal balls from the cultures represent colonies or centers of reproduction. They are exactly like the spirochetal balls or conglomerations seen in congenital syphilis and dementia paralytica. The conclusion seems justified that in these syphilitic diseases with very active spirochetal reproduction the conglomerations represent the same features of reproduction as do those in artificial solid mediums. Cultures in fluid mediums may show similar conglomerations, but usually the colonies are smaller and do not contain such large numbers of spirochetes. Apparently the physical resistance to the expansion of spirochetes during their reproduction is lower in fluid mediums than in solid or semisolid mediums. Therefore, numerous small conglomerations may be seen in fluid mediums, while in solid or semisolid mediums larger but less numerous colonies may be produced. Another feature of this type of reproduction of spirochetes in solid mediums has been observed: liquefaction of the medium in the immediate neighborhood of the spirochetal reproductive colony goes on. It may be of interest to note that the reproductive colonies

in tissues from syphilitic patients usually show the same phenomenon of circumcolonial liquefaction. (Compare figure 2, an artificial culture, and figure 1 *C*, a conglomeration of spirochetes in cortex from a patient with dementia paralytica.)

4. Similar conglomerations of leptospiras were seen in the kidneys of rats naturally infected with these organisms. These conglomerations were found only in the renal tubules. The glomeruli and other parts

Fig. 2.—Culture of the spirochetes of relapsing fever (Borrelia duttoni) in coagulated serum; × 1,000. Note the conglomeration and the pericolonial liquefaction.

of renal structures did not show any leptospiras. In some places the masses were so large as to occlude the lumen completely; in other places the organisms were seen between the epithelial cells of the tubules (fig. 1 *B*).

In silver preparations the inner parts of the large leptospiral conglomerations showed a light yellow color, while the outer parts were formed by black leptospira. So the same picture as seen in spirochetal

colonies in syphilis and relapsing fever appears also in leptospiral infections. One difference was noted, however: The colony has a hollow center which corresponds to the lumen of the renal tubule. Apparently the organisms are washed off by the flow of urine.

The conglomerative appearance of colonies in tissues and the appearance of similar colonies in solid artificial culture mediums seem to indicate that spirocheal reproduction under certain circumstances results in the formation of a characteristic morphologic picture.

The tissue reaction which results from the conglomeration of spirochetes is local miliary necrosis. This type of necrosis is seen in the organs in congenital syphilis (liver, lung, pancreas, adrenal, vascular walls and other tissues) and in the gray matter of the brain in dementia paralytica.

To describe the lesions of congenital syphilis many confusing terms have been used. Some of these are: miliary necrosis, miliary granuloma, miliary abscess-like formation (without liquefaction), miliary syphiloma and miliary gumma. There are at least three different circumscribed histologic appearances in congenital syphilis: miliary abscess-like formation, miliary necrosis and miliary granuloma. The so-called abscesses of Dubois in the thymus in congenital syphilis are necroses with secondary invasion of the necrotic masses by polymorphonuclear leukocytes. Miliary necroses with secondary invasion by leukocytes are also found in other organs in congenital syphilis. In all instances the necrosis is the primary lesion and not, as was formerly believed, necrosis of the central zone of a granulomatous reaction. The necrosis produced by the conglomeration of spirochetes is of a coagulation type. At the outset no formation of granulomatous tissue can be seen in these necrotic areas. Later they may develop into miliary syphilomas, which then may show central necrosis surrounded by granulomatous tissue. But such a sequence has not definitely been established. At any rate, miliary and submiliary necrosis with or without the secondary invasion of leukocytes is the histologic parallel of spirochetal conglomerations. This was first found by Benda [8] and since then by numerous other investigators. In the hypophysis pure necroses were seen, also.[14] In 1928 Schneider [15] stated that the relationship of this type of necrosis in the pituitary to the spirochetes had not been examined.

The anterior lobe of the pituitary in 1 of the 3 cases of congenital syphilis in my material showed spirochetal conglomerations in silver

14. Schmidt, M. B.: Verhandl. d. deutsch. path. Gesellsch. **6**:207, 1903. Simmonds, M.: Dermat. Wchnschr. (supp.) **58**:104, 1914. Schmidt, P.: Centralbl. f. allg. Path. u. path. Anat. **34**:466, 1923. Kraus, cited by Schneider,[15] p. 233.

15. Schneider, P.: Verhandl. d. deutsch. path. Gesellsch. **23**:177, 1928.

preparations and miliary necroses in sections stained with hematoxylin and eosin. In order to establish the relationship of the spirochetal colonies to the miliary necrosis, paraffin sections stained by the gum mastic silver method [7] were made free of silver by diluted nitric acid, washed, and then stained with hematoxylin-eosin or cresyl violet. By this method the areas of miliary necrosis have been identified with the spirochetal conglomerations (fig. 1 *D* and *E*). Serial sections, one

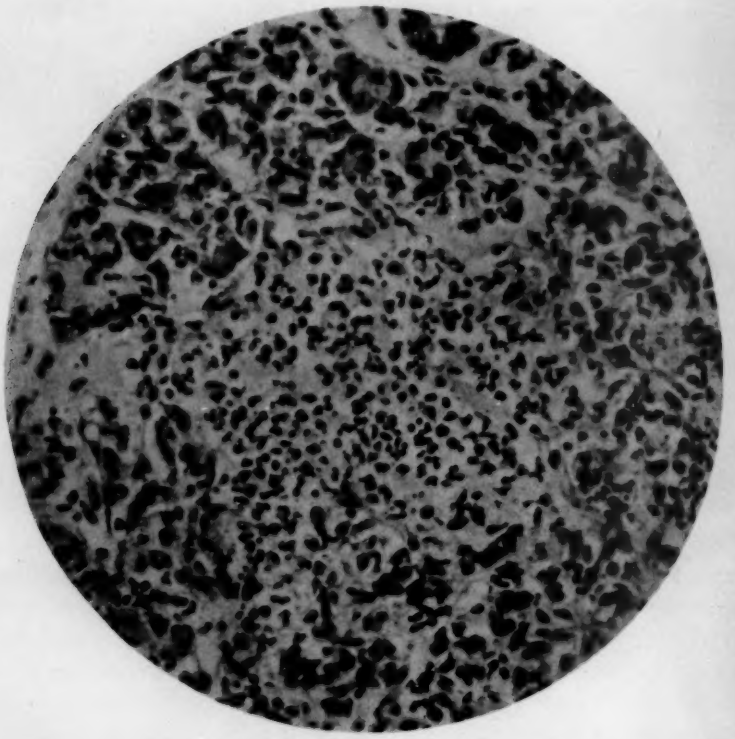

Fig. 3.—Submiliary necrosis in the anterior lobe of the pituitary; same section as figure 1 *D*, made free of silver and stained with cresyl violet; × 300. Note invasion by polymorphonuclears.

stained with hematoxylin-eosin, the next stained by the gum mastic silver method, showed the same relationship between spirochetal conglomerations and miliary necroses. Occasionally an invasion of polymorphonuclears into the necroses or conglomerations was found (fig. 3). This represents a transition toward abscess formation.

Primary necroses in organs in cases of congenital syphilis and in the brain in dementia paralytica are the immediate consequence of spiro-

chetal conglomerations, which themselves result from the very active reproduction of the causative agent in the tissues of the host.

COMMENT

Miliary necroses in the tissues, as well as massive conglomerations of spirochetes (Benda's conglomerative centers), have been recognized as peculiar and rather characteristic lesions in congenital syphilis. Miliary necroses in the gray matter of the brain are also rather frequently demonstrated in dementia paralytica. In both instances the immediate relationship between these spirochetal conglomerations and the subsequent necroses has been established. That the miliary necroses or transitional stages between these miliary necroses and abscesses in the pituitary are due to spirochetal conglomerations is a new observation. The spirochetal conglomerations must be considered as reproductive centers; they are colonies of organisms in the living tissues of the host. The reasons for their occurrence are already given. The contention that the conglomerative phenomenon is not a postmortem effect is substantiated by the necrobiotic changes in the tissue at the same place. The histologic necrobioses are immediate intravital consequences of the spirochetal conglomerations. That the spirochetal conglomerations are not simply a part of a terminal stage of the infection may be demonstrated in 1 of the 3 cases of dementia paralytica. Here sudden death occurred, caused by epileptiform attacks, and the autopsy was done shortly after death. The spirochetal conglomerations in this case were the same as those found in the other 2 cases of dementia paralytica. Furthermore, there is no probability that the uniform pictures of spirochetal conglomerations found in many different organs in the same case of congenital syphilis are terminal effects. In dementia paralytica the difference in size and shape of spirochetal colonies indicates various evolutionary stages of spirochetal reproduction.

Reproductive colonies are found only in very acute stages of syphilitic diseases. The structure of these colonies in the tissues has an appearance which is almost identical with that of colonies growing in solid mediums. Furthermore, in the final stages of some spirochetal diseases characterized by the agglomerative phase of spirochetal reproduction (relapsing fever and spirochetosis gallinarum) numerous single *degenerating* spirochetes are almost always found. Degenerating spirochetes are recognized by the presence of spherical granules on one or both ends of the individual organism, by deformed spirals, by rings or loops, by parts fused together or even by isolated granules. Such degenerated forms are not seen in recent conglomerations or in their vicinity, where spirochetes are always rich in number.

In the conglomerative mass the center is the oldest part. The conglomeration grows by apposition of newly formed spirochetes, but the center does not show any signs of definite spirochetal degeneration. There is a difference in color, but this cannot be considered as a definite degenerative phenomenon. In this regard it would be of interest to follow the different histologic changes of the miliary necroses and the parasitologic findings in them at different evolutional periods of disease. In congenital syphilis older types of necroses, which are characterized by the invasion of leukocytes, occasionally are completely free of spirochetes; or the outer periphery may be marked by a circle of black granules and a few single spirochetes while the surrounding tissues contain very numerous diffusely distributed single spirochetes (fig. 1 *D*). Thus at least a mark has been left where the growing center was, and another proof has been found that these conglomerations are really intravital germinative centers.

Finally, it may be asked how this peculiar conglomerative type of reproduction of spirochetes can be explained. In answering this, different factors must be considered:

First, it may be assumed that the reproductive activity of single spirochetes is rapid and that their motility in the tissues is relatively slow.

Second, the tissue resistance may in some way prevent the natural movement of spirochetes, for in fluid mediums massive conglomerations are not seen.

Third, some material of gluelike character may be produced by the spirochetes in earlier periods of growth or by the tissues themselves. Such a material would hold the spirochetes together. The lighter color of the inner parts of conglomerations in silver preparations could be explained in the same manner. But if parts of these inner centers are removed and stained for spirochetes, the organisms appear black; consequently the presence of a gluelike material seems improbable.

Fourth, the difference in argyrophilic affinity between the central and the peripheral parts of spirochetal conglomerations may be explained by a difference in density of the spirochetal mass. The inner parts may be much denser than the outer ones. For this reason the compact inner part may not take the silver salt solution as well as the outer zones. This explains the difference in color but not the specific type of growth in conglomerative masses. At present no explanation for this specific conglomerative type of reproduction can be offered.

SUMMARY

Spirochetal conglomerations seen in tissues in congenital syphilis and in dementia paralytica are compared with the similar appearing

spirochetal colonies in solid artificial culture mediums and with colonies seen in tissues of animal carriers (rats with leptospiras).

Areas of miliary necrosis in congenital syphilis and dementia paralytica are interpreted as the immediate consequence of these reproductive spirochetal conglomerations. One instance is cited in which miliary and submiliary necrosis in the pituitary in a case of congenital syphilis could be shown to be due to conglomerations of spirochetes.

Evidence has been given to indicate that these spirochetal conglomerations are reproductive centers.

Mr. C. Graham Eddy made the excellent photomicrographs.

RELATION OF THE ELASTIC TISSUE IN THE ROOT OF THE AORTA TO THE AORTIC VALVE

INVOLVEMENT OF THIS TISSUE IN SYPHILIS

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The manner in which syphilis of the aorta may involve the cusps of the aortic valve and the characteristic deformities that ensue have been described by Saphir and Scott,[1] Bell and Clawson,[2] Martland[3] and others. The earlier literature was reviewed by Saphir and Scott.[1] It has been the general experience that the usual changes in the leaflets depend largely on alterations in the adjacent wall, especially at the cusps' highest and lateral points of attachment. While syphilitic valvulitis has been described,[4] its occurrence is relatively rare. The free margins and commissural attachments of the cusps are constantly involved, whereas the basal portions are almost always spared, even in advanced cases. Microscopically the cusps show marked fibrous overgrowth and hyalinization[5a] rather than cellular infiltration or increased vascularity. Signs of inflammation, when they occur, are apt to be limited to the lateral borders.[1] Even if one assumed that the scarring of the cusps is the end result of previously active inflammation, it would be surprising indeed that so little vestige of the involvement persists, as the inflammatory character of the changes in the wall of the aorta is generally unmistakable. Moreover, it is extremely uncommon to observe changes in the leaflets of the valve without concomitant involvement of the root of the aorta. Thus, the weight of evidence seems to indicate that the cusps are secondarily and passively altered in much the same fashion as is the intima elsewhere. Primary specific inflammatory changes of syphilitic aortitis involving the intima directly are perhaps just as rare as similar changes in the leaflets of the aortic valve, the superior portions of which, at least, are but appendages of the inner layer of the aorta.

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1. Saphir, O., and Scott, R. W.: Am. J. Path. **3**:527, 1927

2. Clawson, B. J., and Bell, E. T.: Arch. Path. **4**:922, 1927.

3. Martland, H. S.: Am. Heart J. **6**:1, 1930.

4. Richter, A. B.: Am. J. Path. **12**:129, 1936. Šikl, H., and Raska, K.: Časop. lék. česk. **76**:793, 1937; abstracted, Arch. Path. **26**:1072, 1938.

5. MacCallum, W. G.: A Textbook of Pathology, ed. 6, Philadelphia, W. B. Saunders Company, 1936, (*a*) p. 701; (*b*) p. 696.

If these premises are conceded, it remains necessary to account for the frequent discrepancy between the degree of involvement of the root of the aorta and that of the aortic valve. The extent of valvular change does not appear to be directly proportional to any one feature of the lesion, not to the degree of dilatation nor to the amount of medial or intimal scarring nor to the intensity of the inflammation. This fact has been commented on by MacCallum,[5b] who stated: "When the sinuses of Valsalva are affected, the aortic valves are likely to be involved and to undergo analogous changes, but every combination of aortic and valve involvement may occur, and sometimes the valves may remain perfectly delicate and competent when the nearby wall of the aorta is profoundly affected." Although the root of the aorta is one of the commonest and earliest sites of involvement, aortic insufficiency develops in only a relatively small number of cases of syphilitic aortitis (20.0 per cent, Carr;[6] 36.5 per cent, Clawson and Bell;[2] 20.7 per cent, Welty[7]). Another finding which serves to emphasize this occasional disparity is the fact that syphilis of the sinuses of Valsalva may be severe enough to cause stenosis of the orifices of the coronary arteries without damaging the valve.

The appearance of the syphilitic lesion at the time of death can be deceptive, of course, and there is no quantitative way of measuring the intensity of the process. No very accurate information concerning the evolution of the changes can be obtained. It is possible that a rapidly developing and progressive aortitis damages the valve with greater facility than a more slowly evolving or an intermittent one. The possibility remains, however, that there is some variable component in the structure of the root of the aorta which may mediate involvement of the valve by rendering it more likely to occur in some instances and by preventing it in others. Variations in the amount of elastic tissue of the media beneath the valve might be accountable, since only those portions of the cusps beneath which elastic tissue is present are usually affected. The fact that the inflammatory changes frequently fail to extend deeply into the sinuses of Valsalva (Martland[8]) is also an indication that the process may end at the borderline of the media. That variations in the insertion of the media into the sinuses of Valsalva may occur might be suspected from the other minor malformations sometimes seen in this area. The cusps may vary in shape, size and number, and the orifices of the coronary arteries are frequently displaced or have accessory openings.

For these reasons the present study was undertaken in order to discover how much variation in the amount of elastic tissue may occur in

6. Carr, J. G.: Am. Heart J. **6**:30, 1930.
7. Welty, J. W.: Am. J. M. Sc. **197**:782, 1939.

relation to the aortic valve in nonsyphilitic aortas, and whether it is of a sufficient magnitude to shield or to expose the cusps in the event of syphilitic inflammation. A comparable study was made on two groups of syphilitic hearts. One of these consisted of hearts in which the aortic valves were altered and incompetent, and the other, of hearts in which the valves were normal in spite of lesions in the nearby wall.

MATERIAL AND METHODS

Single sagittal, or profile, sections, 2 mm. wide, were cut through the commissure between the right and the left aortic cusp (B N A terminology) of 73 nonsyphilitic adult hearts obtained at autopsies on the Third (New York University) Division of Bellevue Hospital. Hearts showing rheumatic or bacterial endocarditis or obvious congenital malformation were excluded. The blocks were cut so as to extend from 0.5 cm. above the superior margin of the commissure proximally, to include a small portion of the left ventricular muscle. The position of the upper aspect of the commissure was indicated by a transverse knife cut into the intima. The tissue was fixed in solution of formaldehyde U. S. P. (1:10), embedded in paraffin, sectioned at 10 microns and stained by the combined Weigert and Van Gieson method. An attempt was made in each case to obtain a section for microscopic study from the central portion of each block, approximating the midpoint of the commissure as closely as possible.

The commissure at the junction of the right and left cusps was chosen because its distance from the left ventricular musculature was readily measured. The membranous portion of the intraventricular septum is apt to lie proximally to the commissure of the right and posterior cusps, and the anterior leaflet of the mitral valve lies inferiorly to the commissure of the posterior and left cusps. The distance of these two commissures to some fixed point in the subvalvular region, therefore, is not easily obtained. However, for comparison, the commissure of the right and posterior cusps was studied in 22 hearts, and that of the posterior and left cusps in 15. The data from these are presented separately.

The stained preparations were projected at a magnification of 40 and the following measurements made: (1) the distance of the projection of elastic tissue beyond the highest point of the commissure proximally toward the left ventricle; (2) the distance between the highest point of the commissure and the point where the media first begins to narrow; (3) the distance from the highest point of the commissure to the beginning of the left ventricular musculature.

These measurements could be made with considerable accuracy in any given section. The only point about which any indecision occasionally arose concerned the end of the broad media, where it began to taper, since there was sometimes fraying of the elastic fibers in this vicinity. Repeated measurements usually varied within 0.1 cm. The method is also open to criticism on the ground that the processes of fixation probably alter the spatial arrangements somewhat, but all the material was treated in the same manner, and the results should be comparable with one another even though they do not represent the exact in vivo measurements. Other errors were introduced because there was no way of obtaining a section through the exact midpoint of the commissure and because obliquities in cutting could not be avoided. These errors are probably insignificant, however, since measurements made from multiple sections of a number of blocks varied within 0.15 cm.

A series of 45 syphilitic aortas which showed extensive involvement of the root of the aorta in all specimens but definite anatomic evidence of aortic insufficiency in 24 was prepared in a similar fashion. The anatomic criteria for the diagnosis of aortic insufficiency were separation of the cusps at their highest point of attachment, thickening of the free margins, endocardial pockets on the subvalvular portions of the left ventricular wall and dilatation and hypertrophy of the left ventricle. Specimens were not included in which the absence or presence of aortic insufficiency was not obvious.

The measurements obtained from the syphilitic group are open to more serious objections than those from the control group, since the lesion was destroying the very tissue under observation and the position of the commissure was subject to displacement. However, the end point of the elastica and the beginning point of the

TABLE 1.—*Influence of Various Factors on Measurements of the Elastic Tissue at the Root of the Aorta in Relation to the Commissure Between the Right and Left Aortic Cusps*

Factor	Number in Group	Distance Between Commissure and End Point of Tapering Elastica	Distance Between Commissure and Limit of Broad Media	Distance Between Commissure and Left Ventricle
		Mean, Cm.	Mean, Cm.	Mean, Cm.
	73 (total group*)	0.74 (total group)	0.07 (total group)	1.20 (total group)
Sex				
Males	45	0.74	0.07	1.22
Females	27	0.73	0.06	1.15
Age				
25-49 years	23	0.71	0.07	1.16
50-69 years	36	0.75	0.06	1.21
70-85 years	13	0.74	0.08	1.22
Heart weight				
Less than 410 Gm.	33	0.72	0.06	1.17
More than 410 Gm.	33	0.75	0.10	1.20
Body length				
Less than 170 cm.	34	0.71	0.07	1.15
More than 170 cm.	29	0.74	0.09	1.23

* Numerical differences in the totals of the various subdivisions are due to the fact that the data on some subjects were not recorded.

left ventricle could be determined accurately in most instances. Moreover, it was felt that by comparing the results from the syphilitic group with those from the nonsyphilitic group a satisfactory evaluation of the significance of changes in the former could be made, particularly with reference to the position of the commissure. It must be admitted that measurements on the end point of the broad media in the syphilitic group are at best only rough approximations.

RESULTS

Relation of Elastic Tissue to the Commissure Between the Right and Left Aortic Cusps in Nonsyphilitic Aortas.—As shown in table 1, the mean values obtained in 73 aortas indicate that the broad portion of the media ends 0.07 ± 0.009 cm. proximally to the highest point of attachment of the cusps, the media then tapering somewhat irregularly

to the apex of an elongated wedge-shaped extension 0.74 ± 0.010 cm. farther along. From this point to the beginning of the left ventricular endocardium, a mean distance of 0.46 ± 0.017 cm., the wall is composed of dense fibrous tissue devoid of elastic fibers. In individual cases there was considerable variation, but in every instance some elastic tissue extended beyond the upper limit of the commissure. In one instance the media began to narrow 0.30 cm. above the commissure, and in another it extended a scant 0.20 cm. beyond it. In such vessels only a minimal amount of elastic tissue lay beneath the valve, so that changes limited to the elastica could hardly affect the commissure. On the other hand, in one instance the media did not begin to taper until 0.32 cm. beyond the commissure and extended 1.15 cm. before the elastic tissue terminated. In such a case the commissural attachment of the cusps was backed by

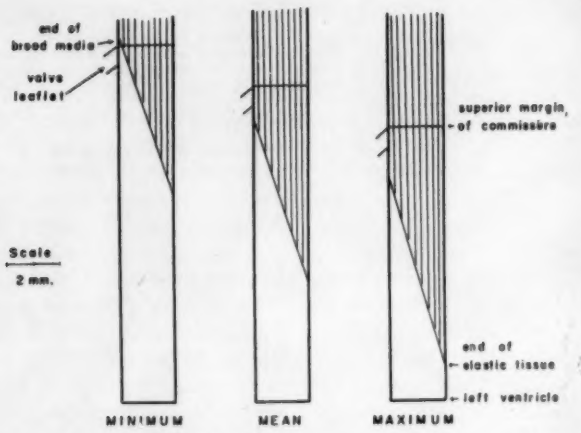

Chart 1.—Relation of the elastic tissue at the root of the aorta to the commissure between the right and left cusps, with standard deviations.

considerable underlying elastic tissue. Similarly, the distance between the end of the elastic tissue and the beginning of the left ventricle varied over a maximum range of 0.67 cm., so that the altered relations of the elastic tissue to the commissure were not based entirely on differences in the position of the latter.

The majority of the measurements fell close to the mean for each point, and symmetric distribution curves could be obtained in which the mean values closely approximated the modes. In chart 1 the relations of the various points under observation to each other are represented diagramatically, and the maximum and the minimum range within the limits of the strandard deviations are shown. It may be seen that even within these restricted borders the amount of medial elastic tissue adjoining the commissure may vary considerably. At the minimal values

only the tapering portion of the media lies beneath the cusps, whereas at the maximal values the broad media extends well beyond their point of origin.

The results were analyzed to see if such factors as sex, body length, age and heart weight played a role in these variations. These factors influence the relative size of the heart and aorta. The measurements recorded here might depend on the size of these two structures. In females and short persons they are generally somewhat smaller than in males and tall persons. The aorta is also known to undergo progressive enlargement with age.[8] Further, the measurements of elastic tissue at the root of the aorta might be secondarily influenced by hypertrophy of the cardiac musculature.

When cases are grouped into these different categories (table 1), the mean values of the elastic tissue measurements are remarkably constant. Perhaps the distance between the upper limit of the commissure and the left ventricle is slightly less in females and subjects less than 170 cm. long, but even this difference is not statistically significant. The relationship of the elastic tissue to the attachment of the aortic valve therefore depends on other individual idiosyncrasies and not on the relative size of the heart and aorta as influenced by the aforementioned factors.

Relation of Elastic Tissue to the Commissure of the Right and Left Aortic Cusps in Syphilitic Aortas.—In table 2 are presented the mean values, with their probable errors, of similar measurements of elastic tissue in 24 syphilitic aortas showing definite evidence of aortic insufficiency and 21 showing no valvular lesion in spite of marked involvement of the sinuses of Valsalva. They are contrasted with the measurements on the control group of nonsyphilitic aortas.

In both syphilitic groups the upper limit of the commissure lies closer to the left ventricle than in the nonsyphilitic group. Although this displacement amounts to only 0.13 and 0.17 cm., respectively, both these differences are greater than four times the square roots of the sums of the squares of the respective probable errors and are therefore presumably significant. The exact mechanism of this displacement is not obvious, but it is not due to separation or other changes in the cusps, since it occurs to the same extent when the valves are not affected. Scott[9] also observed that in syphilis the cusps may be attached several millimeters below the normal site. Perhaps the stretching of the supra-

8. Kaufmann, L.: Zur Frage der Aorta angusta: Ein Beitrag zu den Normalmassen des Aortensystems, Jena, Gustav Fischer, 1919; Veröffentl. a. d. Geb. d. Kriegs- u. Konstitutionspath. **1** (pt. 2):1, 1919.

9. Scott, R. W.: Cardiovascular Syphilis, in Moulton, F. R.: Syphilis, Publication 6, American Association for the Advancement of Science, Lancaster, Pa., The Science Press, 1938, pp. 118-122.

valvular portions of the aorta resulting from inflammatory changes and scarring is more effective on the outer, more pliable coats of the aortic wall than on the more resistant, although thinner, intimal layer.

In any event this displacement alters the relations of the commissure with the elastic tissue of the media in both the incompetent and the competent valvular groups, so that in both the media appears to narrow at a higher point and to terminate a shorter distance beyond the commissure.

If a correction is made for this altered position of the commissure, or if the distance between the end point of the elastic tissue and the left ventricle is taken, the nonsyphilitic group occupies a position intermediate between the two syphilitic ones. That is, the elastic tissue extends less beyond the true commissure and ends a greater distance

TABLE 2.—*Extension of Elastic Tissue Beneath Left and Right Aortic Cusps in Syphilitic Aortas With and Without Aortic Insufficiency*

Group	Number in Group	Distance Between Commissure and End Point of Tapering Elastica	Distance Between Commissure and Limit of Broad Media	Distance Between Commissure and Left Ventricle	Distance Between End of Elastica and Left Ventricle
		Mean, Cm.	Mean, Cm.	Mean, Cm.	Mean, Cm.
Syphilitic aortas with aortic insufficiency.....	24	0.66 ± 0.031	0.016± 0.021	1.07 ± 0.029	0.41 ± 0.020
Syphilitic aortas without aortic insufficiency.....	21	0.45 ± 0.026	−0.08*± 0.023	1.03 ± 0.022	0.58 ± 0.026
Nonsyphilitic aortas.....	73	0.74 ± 0.010	0.07 ± 0.009	1.20 ± 0.013	0.46 ± 0.017

* The broad media ended distal to the upper border of the commissure.

from the left ventricle in syphilitic patients without aortic valve insufficiency than in nonsyphilitic persons. On the other hand, in syphilitic patients with aortic insufficiency the elastic tissue extends farther beyond the original position of the commissure and ends at a point closer to the left ventricle than in nonsyphilitic persons.

When the data on the two syphilitic groups are compared (table 2), it is seen that the elastic tissue in the cases of syphilis with involvement of the aortic valve extended a mean distance of 0.21 cm. farther beyond the commissure and ended 0.17 cm. closer to the left ventricle than in the cases in which the aortic valve was not damaged. Although these differences are perhaps not impressive, they are of sufficient magnitude to be statistically significant. Moreover, in the latter group the media appeared to narrow 0.08 cm. above the commissure, whereas in the group with aortic insufficiency the broad media reached the commissure. As already indicated, this measurement is not very reliable, because of the changes wrought by the syphilitic process, but it does support the impression that less elastic tissue is to be found beneath valves which are

unaltered by the lesion. The significance of these differences is best visualized in diagrammatic form (chart 2).

That the variations in the mean values are not due to the inclusion of a few unusual cases in each group is shown by the curves of the percental distribution of the distances between the end point of the elastic tissue and the left ventricle in chart 3. The curve for the distances in nonsyphilitic aortas (the solid line) is fairly symmetric, and its peak closely approximates the arithmetic mean. All the values for the syphilitic aortas lie within the extremes of the control series. In a major portion of those without aortic insufficiency, 81.0 per cent, the elastic tissue ends a greater distance from the left ventricle than the mean of the nonsyphilitic group. On the other hand, 70.8 per cent of the syphilitic aortas with insufficiency show elastic tissue which extends

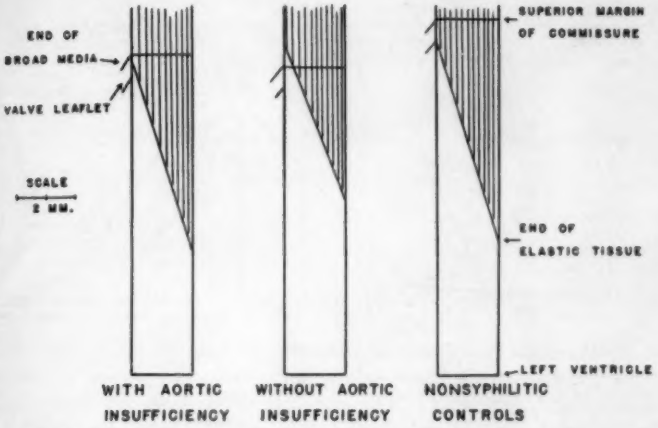

Chart 2.—Mean measurements of the elastic tissue at the commissure between the right and left aortic cusps in syphilitic aortitis with and without aortic insufficiency.

closer to the left ventricle than that in the control group. The frequency distribution shows a greater difference between the two types of syphilitic aortas than do the mean values, for the greatest number of the values for the syphilitic aortas without insufficiency fall near 0.78 cm. and the greatest number of those for syphilitic aortas with insufficiency lie near 0.33 cm., a discrepancy of 0.45 cm. The distribution shown by the curves suggests that when the syphilitic process involved aortas in which the elastic tissue ended farther than 0.46 cm. from the left ventricle, aortic insufficiency was less apt to develop than when it attacked those in which the elastic tissue extended closer to the left ventricle than this.

Amount of Elastic Tissue Beneath Each of the Three Aortic Valve Commissures and Its Relation to the Degree of Involvement of Each in

Syphilitic Aortas with Aortic Insufficiency.—The extension of elastic tissue beneath each commissure varies in the same and different persons. Usually there is a significantly longer projection at the commissures between the right and posterior cusps and between the posterior and left cusps than at the one already discussed, namely, between the right and left cusps. If this is so, and if the amount of adjacent elastic tissue plays a role in determining the extent of involvement of the valve in syphilis of the root of the aorta, it might be expected that a correlation between the two is demonstrable.

In table 3 it may be seen that the elastic tissue at the attachments of the right and posterior cusps and at the attachments of the posterior and left cusps extends, on the average, 0.21 and 0.31 cm., respectively, farther beyond the highest point of the commissure toward the left ventricle than it does at the attachment between the right and left cusps.

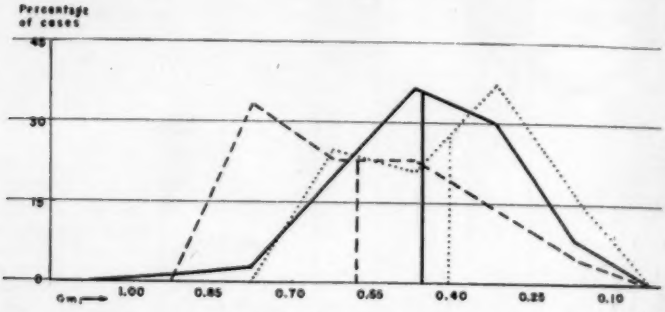

Chart 3.—Percental distribution of distances between the end point of the elastic tissue of the aorta and the left ventricle in nonsyphilitic aortas and syphilitic aortas with and without insufficiency. The distances in nonsyphilitic aortas are represented by the solid line (73 cases, mean 0.46 ± 0.017 cm.); the distances in the syphilitic aortas with insufficiency by the dotted line (24 cases, mean 0.41 ± 0.020 cm.), and the distances in the syphilitic aortas without insufficiency in the dash line (21 cases, mean 0.58 ± 0.026 cm.). The mean value of each group is indicated by the corresponding vertical line.

Differences in a similar direction but of less magnitude and not statistically valid are shown between the end point of the broad media and the commissure in all three instances. One measurable feature of the syphilitic involvement at each of these three points, the degree of separation of the cusps, is compared. This was done by measuring the width of the gap between the two cusps at their closest point of approximation, by means of a hand lens, to the nearest 0.5 mm. in a series of syphilitic aortas with aortic insufficiency. Differences in the number of observations are due to the fact that some commissures had been excised before this measurement was made. Although this is a crude method of estimating the degree of involvement at each commissure, the data do support

the concept that the distribution of elastic tissue is a factor in the development of commissural changes. The commissures possessing the largest amount of underlying elastic tissue showed the greatest mean degree of separation of the leaflets.

This was further borne out when the degree of involvement of each commissure in the same aorta was compared. The commissure with the least subjacent elastic tissue, that between the right and left cusps, showed the greatest degree of damage in only 20 per cent of 30 syphilitic

TABLE 3.—*Extension of Elastic Tissue Beneath Each Commissure of the Aortic Valve, and Its Relation to the Degree of Involvement of Each in Syphilitic Aortitis with Insufficiency of the Aortic Valve*

	Commissure at Attachment of					
	Left and Right Cusps		Right and Posterior Cusps		Posterior and Left Cusps	
Extension of elastic tissue proximal to commissure in nonsyphilitic aortas	Aortas	Mean	Aortas	Mean	Aortas	Mean
1. Limit of broad media.....	73	0.07 ± 0.009 cm.	22	0.11 ± 0.021 cm.	15	0.16 ± 0.024 cm.
2. End of tapering portion..	73	0.74 ± 0.010 cm.	22	0.95 ± 0.036 cm.	15	1.05 ± 0.042 cm.
Degree of separation of cusps at commissures in syphilitic aortas with involvement of valve.....	42	1.03 mm.	55	1.15 mm.	54	1.39 mm.
Relative involvement of each of the three commissures in 30 syphilitic aortas *						
1. With greatest degree of involvement	6	20.0%	6	20.0%	13	43.4%
2. With least degree of involvement	9	30.0%	7	23.3%	2	6.7%

* Those in which two or more commissures showed an equal amount of involvement are not included.

aortas and the slightest degree in 30 per cent. The commissure possessing the most elastic tissue, the one between the posterior and left cusps, was most affected in 43.4 per cent and least altered in 6.7 per cent. This evidence offers collateral support to the main thesis, that the extent of elastic tissue at the root of the aorta is of some importance in determining whether or not the valve will be liable to involvement in syphilitic aortitis.

COMMENT

The findings indicate that in the normal aorta the media extends proximally to about the upper margin of the commissure and then tapers to a variable length beyond it. Although the magnitude of variations in the extension of the media in this region is scant, it is of some significance because the only point of contact of the cusps with the aortic media is at their upper and lateral attachments.[10] In the central portions

10. Lewis, T., and Grant, R. T.: Heart **10**:21, 1923.

of each sinus of Valsalva the media invariably terminates above the point of origin of the leaflet. Even a difference of a few millimeters in the extent of the media at the commissures can radically alter the relationship of the two. The measurements recorded here do not express completely the extent of this variation. In some hearts the angle formed by the adjacent aortic cusps at their lateral attachments is much more acute than in others. In such instances much more of the valvular attachment has aortic media beneath it. In some cases the media narrows abruptly at the upper limit of the commissure, and only a thin strand continues toward the left ventricle. In others the narrowing is much more gradual so that a fairly thick layer of media lies proximal to the upper limits of the cusps. Such variations alter the effective area beneath which the valve attachments have medial tissue.

It should be stressed that in every case some portion of the media extended at least to the highest point of attachment of the aortic valve. If involvement of the cusps depends secondarily on changes in the media, every aortic valve is potentially vulnerable to damage by syphilitic aortitis, at least to some extent. The severity and distribution of the inflammatory process thus remain the decisive factors in the development of aortic insufficiency. The findings reported here indicate merely that some valves may be much more readily involved than others, owing to variations in the preexisting structure of the valve ring. Whatever the exact mechanism of the changes in the leaflets may be, whether it is an extension of the inflammation from the wall or a process secondary to degenerative changes in the media which in turn are attributable to involvement of the adventitia or vasa vasorum, the fact cannot be ignored that only those portions of the valve beneath which the media is present are usually affected. Thickening of the free margin may well be caused by mechanical factors after aortic insufficiency has been established, according to Martland.[3] In those few instances in which active inflammation of the entire cusp is present and Spirochaeta pallida is demonstrable,[4] the development of aortic insufficiency is obviously more independent of the mode of attachment.

The significance of the differences observed in the elastica of the two syphilitic groups cannot be evaluated with certainty. These differences may be the consequence of variations in the syphilitic lesion and not express variations that existed before the inflammatory process was initiated. If this were so, however, it might be expected that the aortas showing the severest lesions, as indicated by the development of aortic insufficiency, would show the most marked destruction and reduction in elastic tissue. Yet more elastic tissue was found beneath commissures which were altered than beneath those which were not. If anything, the syphilitic process would tend to diminish and obscure the differences between the two syphilitic groups rather than to exaggerate them.

The possibility cannot be ruled out, however, that a certain degree of regeneration of elastic tissue occurs as the lesion progresses with ultimate extension of the elastic tissue beyond its original position. If this occurs at all, it might be more pronounced in the aorta with the severest involvement, namely, that with aortic insufficiency. The difference observed could thus be interpreted as the result of the lesion. The process elsewhere in the aorta, however, does not appear to be associated with any conspicuous degree of elastic tissue growth. There is a progressive loss of elastic tissue, with narrowing of the media, although the other coats are greatly thickened. Moreover, the range of measurements of elastic tissue in the syphilitic aortas was within that of the control series. If regeneration of elastic tissue was a factor in altering the measurements, in no instance did it extend closer to the left ventricle than it sometimes does in normal aortas. The weight of evidence is against the interpretation that secondary regeneration of elastic tissue alters the original distribution of this tissue to any appreciable degree.

SUMMARY

Measurements on the nonsyphilitic aorta reveal that the elastic tissue of the media at the root may project a variable distance proximal to the commissural attachments of the aortic valve cusps and that the lateral attachments of the cusps may show a great deal or very little of the medial coat in the wall of the underlying aorta. These variations do not appear to be related to the size of the aorta or heart and are not influenced by age, sex, body length or heart weight.

Since the lateral attachments of the aortic cusps are chiefly involved in the development of aortic insufficiency due to syphilis, it is pointed out that the degree of valvular damage may depend in part on the extension of the media in this area.

Measurements on the syphilitic aorta support this concept. The elastic tissue of the media is found to end more abruptly at the commissures in cases in which aortic insufficiency has failed to develop than in cases in which the valves are incompetent.

The three commissures in the normal aorta have different amounts of elastic tissue in their walls. Although the relative degrees of involvement of each of the three commissures varies in individual cases, in a series of cases of syphilitic aortitis with aortic insufficiency the average degree of involvement varied directly with the amount of intramural elastic tissue.

The highest point of attachment of the aortic cusps is found to be lower in the syphilitic aorta than in the normal one. This displacement is not related to changes in the leaflets themselves, since it is found when the cusps are still delicate and normally inserted.

MYASTHENIA GRAVIS AND THE THYMUS GLAND

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Recent papers, particularly those of Norris,[1] have again drawn attention to the relation between myasthenia gravis and the thymus. They have pointed out that only 82 autopsies on the disease are recorded in the literature and that in 37 of these a lesion of the thymus was found to be a prominent anatomic feature. Norris emphasized the importance of recording the pathologic observations in all investigated cases of myasthenia gravis.

The association of a tumor of the thymus with myasthenia gravis was first described by Weigert,[2] in 1901. He considered the tumor a lymphosarcoma and looked on the lymphorrhages in the voluntary muscles as metastases. Buzzard [3] contested this view, demonstrating lymphorrhages without apparent involvement of the thymus, and after Marburg [4] lymphorrhages were increasingly regarded as signs of a reactive process in degenerating muscle. Hart [5] suggested that the "tumors" of the thymus were merely different degrees of hyperplasia of the epithelial and lymphoid cells of the gland.

Bell [6] and Norris [1a] reviewed series of cases of myasthenia with regard to thymic involvement, finding it in at least 50 per cent of adequately reported cases. Norris stated that "pathologic changes may be found in the thymus in cases of myasthenia gravis in direct ratio to the care with which they are sought." Thus, none were reported prior to 1901. Starr [7] found them in 28 per cent of a series of 250 cases collected from the literature, Bell [6] in approximately 50 per cent of his 56 cases and Norris [1] in 50 per cent of his further cases. Bell stressed the specific character of the changes in the thymus—a reversion to a fetal type of structure produced by the disproportionate hyperplasia of the epithelial elements, which are relatively inconspicuous in the adult

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From the Department of Pathology of the Johns Hopkins University.

1. Norris, E. H.: (*a*) Am. J. Cancer **27**:421, 1936; (*b*) **30**:300, 1937.

2. Weigert, C.: Neurol. Centralbl. **20**:597, 1901. Laquer, L.: ibid. **20**:594, 1901.

3. Buzzard, E. F.: Brain **28**:438, 1905.

4. Marburg, O.: Ztschr. f. Heilk. **28**:111, 1907.

5. Hart, C.: Virchows Arch. f. path. anat. **220**:185, 1915.

6. Bell, E. T.: J. Nerv. & Ment. Dis. **45**:130, 1917.

7. Starr, M. A.: J. Nerv. & Ment. Dis. **39**:721, 1912.

gland—and pointed out that such an appearance is found almost exclusively in tumors associated with myasthenia gravis. Norris described the condition as a benign tumor in half the cases and as persistent enlargement in half, but put forward the view that this division is artificial and that both classes represent degrees of hyperplasia, extreme and moderate, respectively.

Obiditsch [8] studied histologically a series of 9 thymus tumors and claimed that those composed predominantly of small round cells were more often associated with symptoms of myasthenia than were epithelial and malignant types.

Meggendorfer [9] and Meister [10] reported cases of a metastasizing growth of the thymus associated with myasthenia gravis, but such cases are extremely rare; thus Meggendorfer's case was at that time the only one of 60 recorded cases of malignant tumor of the thymus in which the tumor was associated with myasthenia.

Schumacher and Roth [11] and Harerer [12] reported cases in which improvement of myasthenia followed extirpation of a tumor of the thymus, the operation in the first case having apparently been performed by Sauerbruch. Beretvás [13] had no success in the treatment of the disease by irradiation of the thymus, but Stern [14] reported improvement obtained by this means.

Recently Adler [15] claimed that he had produced myasthenia-like symptoms of exhaustion in dogs by transplanting thymus tissue or by injecting a watery extract of juvenile thymus and that the animals' symptoms were rapidly relieved by injections of prostigmine. This work is as yet unconfirmed, and the account is lacking in detail. Nevin [16] claimed that injections of a commercial extract of thymus had produced aggravation of symptoms in some cases of myasthenia gravis and pointed out that administration of thyroid extract is followed by a temporary exacerbation which may be due to stimulation of the thymus.

Lièvre [17] summarized the evidence for the importance of the thymus in myasthenia gravis thus: 1. A tumor or hypertrophy of the thymus

8. Obiditsch, R. A.: Virchows Arch. f. path. Anat. **30**:319, 1937.

9. Meggendorfer, F.: Ann. d. städt. allg. Krankenh. zu München **13**:116. 1908.

10. Meister, M.: Klin. Wchnschr. **15**:1389, 1936.

11. Schumacher and Roth: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **25**:746, 1913.

12. von Harerer, H.: Arch. f. klin. Chir. **109**:193, 1918.

13. Beretvás, L.: Riforma med. **41**:771, 1925.

14. Stern, R.: Wien. klin. Wchnschr. **50**:321, 1937.

15. Adler, M.: Arch. f. klin. Chir. **189**:579, 1937.

16. Nevin, S.: J. Neurol. & Psychiat. **1**:120, 1938.

17. Lièvre, J. A.: Presse méd. **44**:991, 1936.

is frequently found in myasthenic patients, and the tumor appears to be a specific lesion. Microscopic tumors may easily be missed at autopsy. 2. The variability of the lesion (hyperplasia, circumscribed tumor) of the thymus applies also to other endocrine disorders. 3. While the diffuse hyperplasia of the gland could legitimately be considered a part of the general proliferation of lymphoid tissue common in this disease, such an explanation seems unlikely in the case of a circumscribed tumor. Lièvre concluded that there is a relation between the thymus gland and myasthenia gravis and that the thymic condition is probably not a non-specific reaction but either causal or concomitant. He recommended roentgen investigation, followed by irradiation or surgical removal.

REVIEW OF FIVE CASES

The pathologic observations in the 5 cases of myasthenia gravis encountered in 16,300 autopsies at the Johns Hopkins Hospital will now be summarized, only positive evidence being given:

CASE 1.—A white man aged 35 had a twelve month history of increasing weakness of the muscles of mastication and respiration. His death was due to respiratory failure.

The autopsy report was: "A clinical history of myasthenia gravis; a tumor nodule in the region of the thymus; a stone in the pelvis of the left kidney; hydronephrosis; pulmonary edema; localized bronchopneumonia; old tuberculosis of the bronchial nodes."

The thymus was fatty and not noticeably enlarged but showed two strips of tissue extending about one third of the way down over the parietal pericardium. Embedded in the midportion of the left lobe was a small firm nodule, measuring 2 by 1.5 by 1 cm., well encapsulated, elliptic and sharply defined from the surrounding normal thymus tissue.

On section this was grayish red and showed small blood-filled spaces; it had a gross appearance similar to the surrounding thymus tissue but was more compact in its arrangement and contrasted sharply with neighboring lymph nodes, which were pigmented and in some instances caseous.

Histologically, the surrounding tissue, stretched around the tumor in isolated patches embedded in loose areolar tissue; it showed the normal structure of the thymus gland with well marked Hassall's corpuscles. The tumor itself was well defined and trabeculated by a connective tissue capsule; it was vascular and was composed mainly of lymphoid cells, with some hyperplasia of the epithelial elements, especially at the periphery (fig. 1 *A* and *B*).

There were no Hassall's corpuscles in the tumor, and the epithelial cells showed no signs of the compression or tension which flattens them in the normal thymus, but had an indistinct cell body and a plump, vesicular nucleus. They extended into the substance of the tumor in a network quite distinct from the masses of lymphoid cells.

Lymphorrhages were present in the voluntary muscles but were rare, being found in only 1 of 18 sections. The thyroid gland contained a distinct excess of colloid, and many alveoli were lined with low, flat epithelium. The other endocrine glands showed no abnormality.

CASE 2.—A white man aged 45 had an eight month history of increasing weakness of the muscles of mastication, phonation, deglutition and ocular movement. Death occurred from respiratory failure.

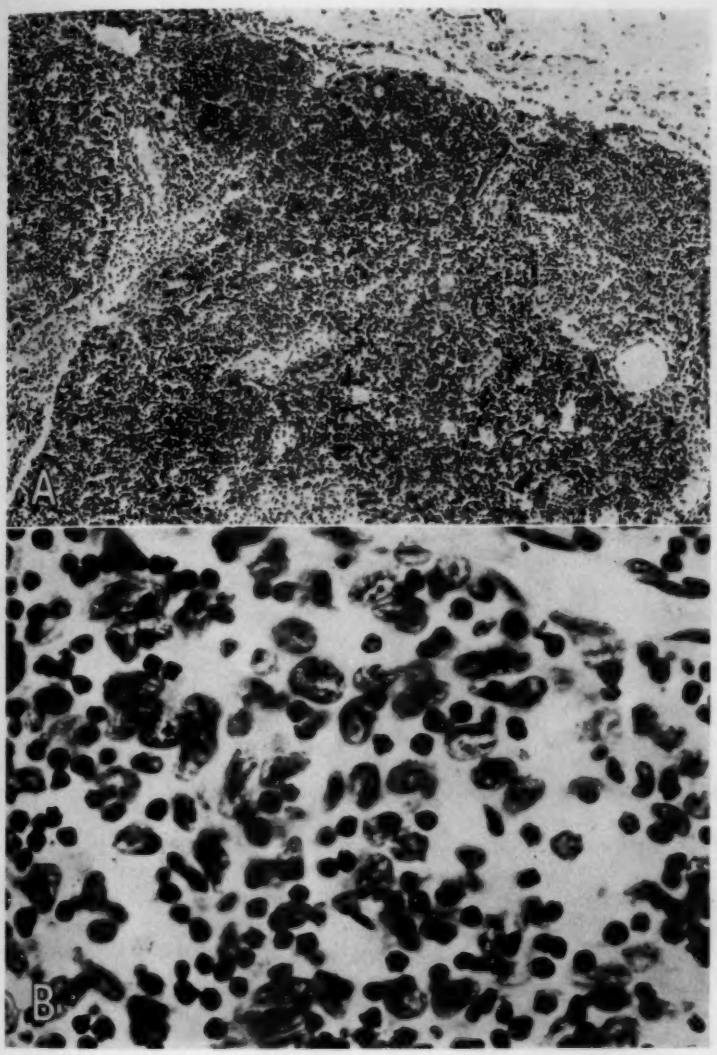

Fig. 1 (case 1).—*A*, thymus tumor; × 90. *B*, same tumor, showing epithelial cells; × 670.

The diagnosis at autopsy was: "myasthenia gravis; persistent thymus; bronchitis; bronchopneumonia; fibrous nodule in the thyroid; fibrous orchitis; hemangioma of the liver; lymphangioma of the spleen; accessory spleen."

There was a bilobed thymus, each lobe measuring 7 by 3 by 1.5 cm., the gland weighing 60 Gm. in all. There was no localized tumor, and histologically the gland consisted of scattered small foci of lymphoid cells embedded in areolar tissue. Some of these foci contained recognizable germinal centers; others, Hassall's corpuscles, which were sometimes calcified. In several places large epithelial cells which inconspicuously surrounded these foci had proliferated, forming small sheets of clear cells, with prominent nuclei, which looked very much like stratified squamous epithelium.

No lymphorrhages were found in the many sections of muscle taken; the thyroid gland was normal except for a hyaline fibrous nodule similar to that seen in the scarred testicles. There was no other abnormality of the endocrine system.

The lungs disclosed, in addition to acute lobular pneumonia, numerous collections of lymphoid cells, particularly around the small blood vessels, and sometimes these showed recognizable germinal centers.

CASE 3.—A white woman aged 27 suffered for four years with slowly progressive weakness of the ocular muscles and of the muscles of mastication and deglutition. She improved when treated with ephedrine, but finally respiratory failure responded only temporarily to prostigmine and was fatal.

The diagnosis at autopsy was "myasthenia gravis; lobular pneumonia; persistent thymus; focal necroses in the adrenal cortex."

The thymus was a rather flat sheet of tissue, measuring 6 by 6 by 1 cm. and weighing 30 Gm. Histologically the gland was uniformly normal, being composed of small round cells with well marked Hassall's corpuscles and with only an occasional suggestion of any differentiation into peripheral and central zones.

There was some scattered necrosis in the fasciculate and glomerular zones of the adrenal cortex in both glands, the cells being replaced by a pink formless material.

No lymphorrhages were found, and the thyroid gland and other endocrine organs were normal.

CASE 4.—A white man aged 63 said that for six months he had had increasing muscular weakness, with improvement on administration of prostigmine, but that gradually this treatment lost effect. There was terminal respiratory distress, and the patient died despite the administration of prostigmine and treatment in the Drinker respirator.

The diagnosis at autopsy was: "myasthenia gravis; lymphocytic infiltration in the muscles; a scar with fibrous adhesions at the apex of the upper lobe of the right lung; pulmonary edema; peculiar fibrosis of the leaflets of the mitral valve; fatty infiltration of the liver; moderate generalized arteriosclerosis with arteriosclerotic changes in the kidneys; calcified prostatic concretions; atrophy and scarring of the testis."

The thymus was not identified. Lymphorrhages were frequent in the voluntary muscles but only rarely were they perivascular. The thyroid gland was normal, and the only other abnormalities were those already summarized.

CASE 5.—A colored man aged 34 had marked exophthalmos of long standing and a doubtful history of symptoms of exophthalmic goiter. For eight months he had noted increasing weakness of the musculature of the head and neck. He was maintained for two months on prostigmine, which he took irregularly. He died in respiratory failure despite the use of prostigmine and the Drinker respirator.

The diagnosis at autopsy was "myasthenia gravis; a tumor of the thymus with remnants of normal thymus tissue; widespread small areas of lymphorrhagia in the muscles and elsewhere; hyperplasia of lymphoid nodules, especially in the pharynx and larynx; colloid goiter; lobular pneumonia."

The thymus weighed 70 Gm. and was the size and shape of a bantam's egg (fig. 2). Most of the gland was taken up by a large tumor, 5 by 3 by 6 cm., with a little normal thymus tissue at the lower pole. The tumor was divided by fine bands of fibrous tissue into lobules of varying size. These contained a mixture of small lymphoid cells and a network of large, ill defined, pale epithelial cells with vesicular nuclei (fig. 3*A*). The proportion of these two kinds of cells varied considerably, and in some areas the "epithelial" elements formed solid blocks of tissue (fig. 3*B*), while in other lobules they were diffusely scattered among the round cells.

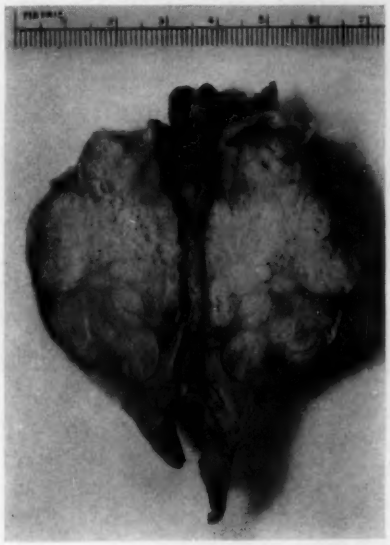

Fig. 2 (case 5).—Thymus tumor.

The lower pole of the tumor was covered by a thin layer of thymus tissue, which was pink in the gross as opposed to the creamy tumor, and was histologically similar to normal thymus though relatively poor in Hassall's corpuscles.

The lymphorrhages were widespread, were perivascular and were found in the adrenal and in the epiglottis, as well as in the pharyngeal, ocular, intercostal and sternocleidomastoid muscles and the diaphragm (fig. 3*C*). In the ocular muscles there were, in addition, scattered areas of atrophy, where the muscle fibers had almost disappeared, leaving what appeared to be an empty fibrous framework (fig. 3*D*). The general lymphoid hyperplasia spared the abdomen and affected only the regions above the diaphragm. The tonsils were huge and hyperplastic, and the pharyngeal and laryngeal follicles extended continuously down into the piriform sinuses and larynx. There was a well marked colloid goiter, which also showed great lymphoid infiltration and some germinal centers.

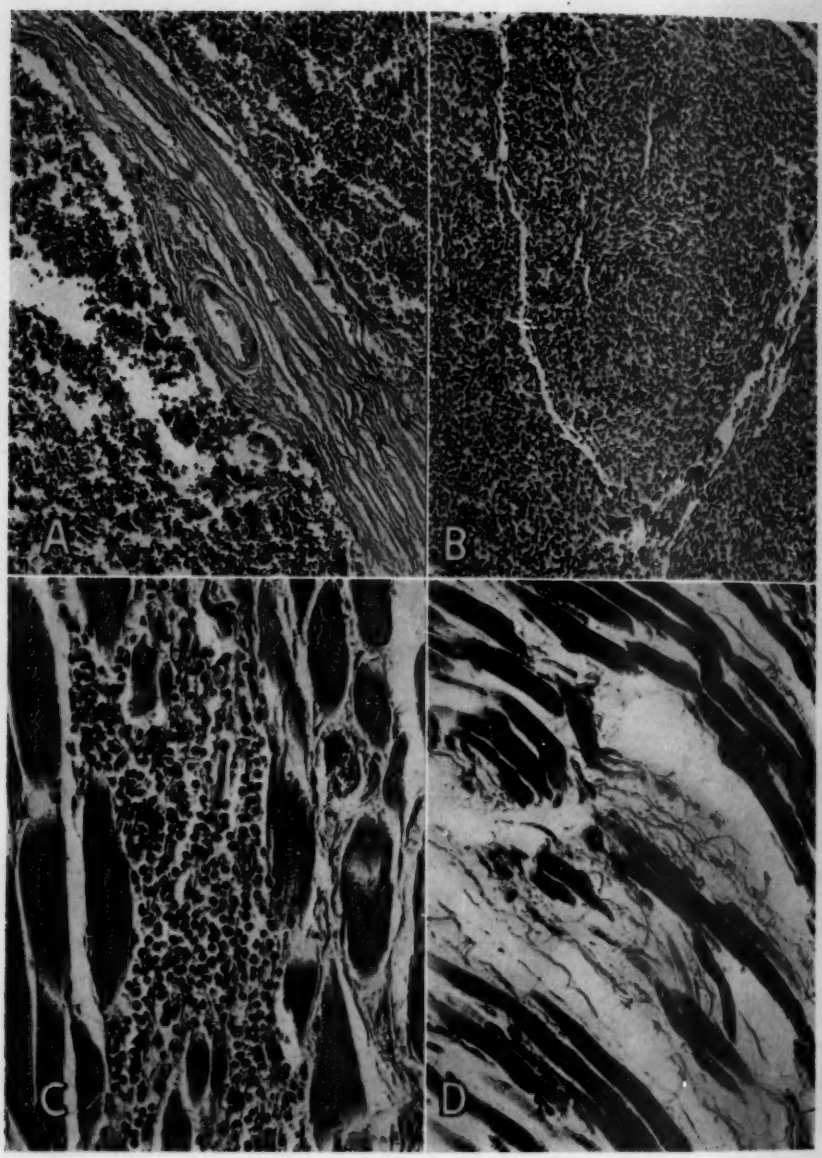

Fig. 3 (case 5).—*A*, thymus showing areas consisting predominantly of lymphoid and epithelial cells, respectively; × 100. *B*, area of tumor composed of solid blocks of epithelial cells; × 100. *C*, typical lymphorrhage from the diaphragm; × 200. *D*, focal areas of atrophy in ocular muscle; × 200.

COMMENT

Of the 5 cases of myasthenia gravis reported here, 2 showed an encapsulated tumor of the thymus gland with remnants of normal thymus outside the capsule; 2 showed a persistent thymus gland, with well marked peripheral epithelial hyperplasia in 1 case; and in 1 case the thymus was not identified.

This brings the number of autopsies in such cases reported in the literature to 87, in 41 of which the lesions in the thymus were found to be a prominent anatomic feature.

It is interesting that in the present 5 cases abnormalities in the thymus were more common pathologically (4 cases) than the characteristic lymphorrhages, which are the accepted criteria of a pathologic diagnosis of the disease, though often difficult to find in clinically indisputable cases.

As regards the role of the thymic condition in the genesis of the disease little can be added. Injection of extracts prepared from such tumors might assist in the elucidation of the problem. In the meantime it seems that careful roentgenologic examination of myasthenic patients is indicated in an effort to diagnose thymic involvement and that irradiation and surgical removal should be essayed in a disease which, despite recent therapeutic advances, is otherwise so lethal in its effects.

SUMMARY

The literature dealing with the relation of the thymus to myasthenia gravis is reviewed, and 5 further cases in which autopsies were done are described. In 2 cases an encapsulated tumor of the thymus was found associated with remnants of normal thymus; in 2, a persistent thymus was observed, with marked peripheral epithelial hyperplasia in 1 case: and in 1 case the thymus was not identified.

This brings the number of reported cases of myasthenia gravis in which autopsy was done to 87, in 41 of which distinct anatomic lesions of the thymus were found.

It is suggested that patients with myasthenia gravis be subjected to a careful roentgen investigation in an effort to diagnose thymic involvement, and that irradiation and surgical removal be essayed more often in the treatment of this disease with so unfavorable a prognosis.

ADDITIONAL OBSERVATIONS ON POSITIVE AND NEGATIVE CHEMOTAXIS

EXPERIMENTS WITH A MYXOMYCETE

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In a recent paper [1] it was reported that negative chemotaxis could be induced in polymorphonuclear leukocytes by certain chemical substances (silicates) and, in some experiments, by a strain of hemolytic streptococci. Negative chemotaxis, i. e., the reaction through which leukocytes are caused to move away from bacteria or other bodies, may well be an important factor in infection, one tending to hinder recovery. This subject has not received the attention accorded to the opposite phenomenon, positive chemotaxis, and requires further investigation. But before continuing the studies on negative chemotaxis in leukocytes, it seemed desirable to obtain broader knowledge as to the occurrence of this reaction in living cells generally. Through observations on a different and perhaps more primitive type of organism it was hoped that negative and positive chemotaxis would appear in better perspective.

The plasmodium of Physarum polycephalum, a myxomycete [2] (slime mold), was selected for the present study. It shows the same type of locomotion (i. e., ameboid) as the leukocytes, and, like the latter, displays both positive and negative chemotaxis. This organism proved suitable for some experiments for which the leukocyte, on account of its small size, vulnerability and environmental requirements, is less well adapted. Experiments were planned with the myxomycete to answer the following questions: What types of chemical substances induce, respectively, positive and negative chemotaxis? Is the positive or negative reaction dependent on the nature of the substance tested or, as has been proposed,[3]

From the Department of Pathology of the University of Pennsylvania Medical School.

This investigation was aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. McCutcheon, M.; Coman, D. R., and Dixon, H. M.: Arch. Path. **27**:61, 1939.

2. It is interesting to recall that myxomycetes were used by Stahl (Bot. Ztg. **42**:145 and 161, 1884) in some of the earliest experiments in chemotaxis.

3. Wells, H. G: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

on the concentration? How does a cell or organism act when it alters its response from positive to negative? Is its behavior qualitatively different, or is a negatively reacting cell the mirror image of one reacting positively?

MATERIAL

The myxomycete, or slime mold, is a naked mass of protoplasm, a syncytium, which occurs naturally in such an environment as the bark of a tree, where it seeks food by an ameboid type of locomotion and where it may attain a size of several centimeters. Its large size permits experimental observations to be made with the naked eye; or minute pieces may be cut off and observed with the microscope.[4] Both these methods have been employed in the present experiments. The culture of Physarum polycephalum was supplied by Prof. W. Seifriz, of the department of botany. This is an orange-colored organism which grows readily in the laboratory, cultures being maintained on wet filter paper sprinkled with dry oatmeal.

METHOD

Observations with the naked eye were made on plasmodia placed in Petri dishes containing 2 per cent agar in distilled water. Disks were cut out of the gelled agar, one in the center and one or more at the periphery. Into the central well thus formed was poured agar containing oatmeal, and into the other wells, agar containing the substance to be tested for its chemotactic effect. The mold was planted on the central disk, and the direction of migration in relation to the disks of test substance was noted during the following eighteen hours.

The different types of reaction of the mold to test substances are shown in figures 1 to 3. Figure 1 illustrates an indifferent reaction (absence of chemotaxis). The mold has extended in all directions and has evidently been neither attracted nor repelled by the test substance in the disks near the edge of the plate. Figure 2 illustrates positive chemotaxis. The organism has migrated from the center to both disks containing the test substance. Figure 3 illustrates negative chemotaxis and is the opposite of figure 2, since, instead of moving toward the disks containing the test substance, the mold has avoided them.

CHEMOTACTIC EFFECTS OF SUGARS, ACIDS AND ALKALIS

By the method just outlined, the chemotactic effects of diffusible substances are readily tested. It is these substances—sugars, acids, alkalis, salts, present everywhere, taking part in all sorts of cellular processes—that, because of their rapid diffusibility, are especially difficult to test with leukocytes. The diffusion of acids and alkalis through the agar jelly may be observed by adding an indicator, such as bromthymol blue, to the agar.

A summary of results obtained with these diffusible substances is given in the table. Dextrose induced positive chemotaxis over a wide

4. Such small pieces in appearance and response are quite comparable to leukocytes or other naked cells endowed with ameboid movement. The suitability of the slime mold for other types of investigation is obvious. For instance, in studies of injury and repair one might find in this a particularly favorable material.

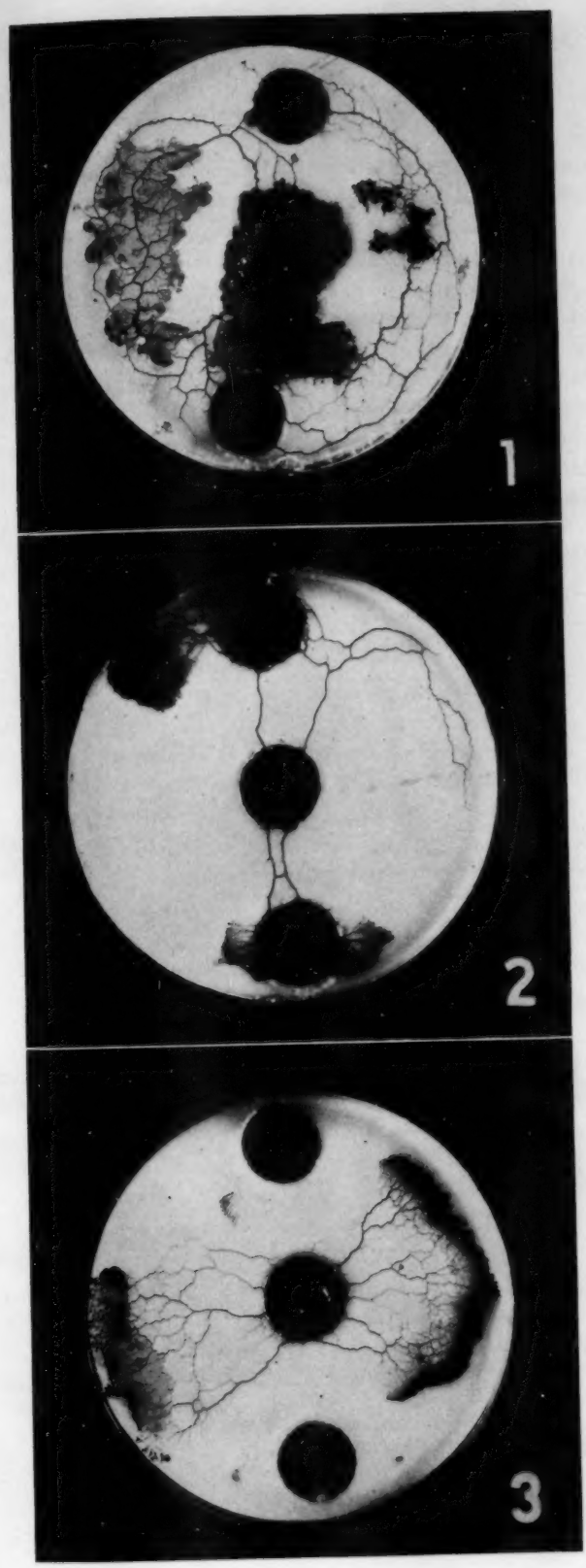
EXPLANATION OF FIGURES 1, 2 AND 3

Photographs are shown of myxomycetes on agar in 100 mm. Petri dishes. In each plate are three black circles, the central one of which represents a disk containing nutrient substance, on which the mold was planted. The peripheral disks contain the substance to be tested for chemotactic effect. The plasmodium appears as irregular gray sheets and filaments.

Figure 1 shows indifferent chemotaxis. The substance tested was sodium chloride. The mold has moved in all directions and shows no orientation to the test substance.

Figure 2 shows positive chemotaxis. The test substance was dextrose. The plasmodium has moved from its original central position to both disks containing the test substance and is seen nearly to surround these disks.

Figure 3 shows negative chemotaxis to a silicate (Lloyd's reagent). The plasmodium, in moving from the center to the periphery of the plate, has avoided the two disks containing the test substance.



Figures 1, 2 and 3

range of concentrations, from hundredth-molar to molar. The reaction was positive in 42 of 44 plates. Saccharose, it is interesting to observe, caused no chemotactic response in any of the concentrations used. The mold moved toward the sugar in 13 plates and away from it in 17, but generally the displacement toward or away from the test substance was slight, and therefore it is concluded that this organism is indifferent to saccharose. Thus the mold is able to discriminate between two closely related sugars. The chemotactic effect of different sugars apparently varies with the species of slime mold.[5]

The effect of hydrogen and hydroxyl ions was tested, using hydrochloric acid, sulfuric acid, acetic acid and sodium hydroxide. As seen in the table, all these substances in concentrations of tenth molar or

Chemotactic Reactions of Myxomycetes to Substances Diffusing Through Agar Jelly Contained in Petri Dishes *

	Preparations of Myxomycetes Reacting to Given Molar Concentration of Substance																	
	0.01		0.02		0.05		0.1		0.2		0.5		1.0		2.0		4.0	
Test substance.....	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Dextrose.....	4	0	5	0	5	0	..	..	8	2	10	0	10	0	..	..	..	..
Sucrose.....	..	..	..	..	..	..	..	..	2	3	2	3	6	4	2	3	1	4
Hydrochloric acid....	2	3	..	..	..	..	0	19	..	..	..	..	..	..	..	..	..	..
Sulfuric acid.....	3	3	..	..	..	..	0	6	..	..	0	6	0	6	..	..	..	..
Acetic acid.....	4	4	..	..	..	..	0	6	..	..	0	6	0	6	..	..	..	..
Sodium hydroxide....	2	3	..	..	..	..	1	5	0	10	0	12	..	..	..	..	..	..
Sodium chloride.....	..	..	..	..	..	..	7	7	..	..	..	..	..	..	..	..	..	..

* Results were recorded about eighteen hours after planting. A positive (+) reaction indicates movement toward the test substance; a negative (−) reaction, movement away from it. The mold was attracted by dextrose in all the concentrations used; it was indifferent to sucrose. Acids and alkalis in concentrations of tenth molar or greater produced negative chemotaxis.

above produced negative chemotaxis; lower concentrations had no effect. Combining the results obtained with the various acids, one finds that the mold was repelled in 55 plates and attracted in none. A similar effect was obtained with sodium hydroxide, the chemotaxis in 1 plate being positive and that in 27 negative, with concentrations of tenth molar or higher. That these results were probably due to hydrogen and hydroxyl ions, respectively, rather than to sodium or chlorine ions or to osmotic effects is seen in the indifferent response to tenth molar sodium chloride: in 7 plates chemotaxis was weakly positive and in 7 weakly negative.

It is concluded from these experiments that both hydrogen and hydroxyl ions when in adequate concentration produce negative chemotaxis in Physarum polycephalum. This result is in general agreement with those reported by Strange [6] and Emoto [5] with other species of

5. Emoto, Y.: Proc. Imp. Acad., Tokyo **8**:460, 1932.
6. Strange, B.: Bot. Ztg. **48**:107, 1890.

slime mold and by Jochims [7] with leukocytes. These workers, however, reported positive chemotaxis to certain weakly acid solutions, a result not duplicated in the present experiments. The effect of hydrogen and hydroxyl ions may be summarized by saying that both cause repulsion, the least repelling concentration for some organisms being somewhat on the acid side of neutrality.

In addition to the repelling effect of high concentrations of hydrogen and of hydroxyl ions on both myxomycetes and leukocytes, other points of similarity in the responses of the two types of organisms have been observed. Thus both are repelled by a silicate (Lloyd's reagent).[1] Also, like myxomycetes, leukocytes are reported to be attracted by certain sugars.[8] Whether they react similarly to other substances must be decided by further experiments.

EXPERIMENTS WITH THE MICROSCOPE

The slime mold is excellently adapted to microscopic observation, and much information has been obtained by low power magnification. A small piece of the mold, about 0.5 mm. long, is snipped off with fine scissors and placed in a thin film of water on a glass slide. The slide is inverted over a small dish to form a moist chamber.

As seen under low power magnification, after a period of quiet, streaming of the protoplasm begins. The streaming has the remarkable characteristic of rhythmicity: the protoplasm flows for many seconds—often 40 to 60—in one direction, and then reverses and flows for approximately the same time in the opposite direction. (For detailed study of protoplasmic streaming see Vouk [9] and Seifriz.[10]) As locomotion develops, the rhythm of protoplasmic streaming alters; the flow toward the advancing pseudopod continues longer than the reflux in the opposite direction.

If an attracting substance, such as a fragment of oatmeal, is placed a few millimeters from the mold, a broad pseudopod is thrust out toward it. The protoplasm in the pseudopod appears to become more liquid, as judged by the turbulent motion of the protoplasmic granules. From the front of the pseudopod are thrust out clear blister-like extensions of protoplasm which, from the rapidity of their formation and flow, appear to have relatively low viscosity. In a few seconds the granular cytoplasm flows into the clear processes, and thus the pseudopod rapidly advances. The organism now assumes a triangular shape, with the broad

7. Jochims, J.: Arch. f. d. ges. Physiol. **216**:611, 1927.

8. Chambers, R., and Grand, C. G.: J. Cell. & Comp. Physiol. **8**:1, 1936.

9. Vouk, V.: Denkschr. d. k. Akad. d. Wissensch. Math.-naturw. Kl. **88**: 653, 1913; cited by Kisser and Metzner: Tabulae biol. **4**:471, 1927.

10. Seifriz, W.: Science **88**:21, 1938.

pseudopod in advance and a narrow tail behind. The organism in this state of activity looks much like a macrophage.

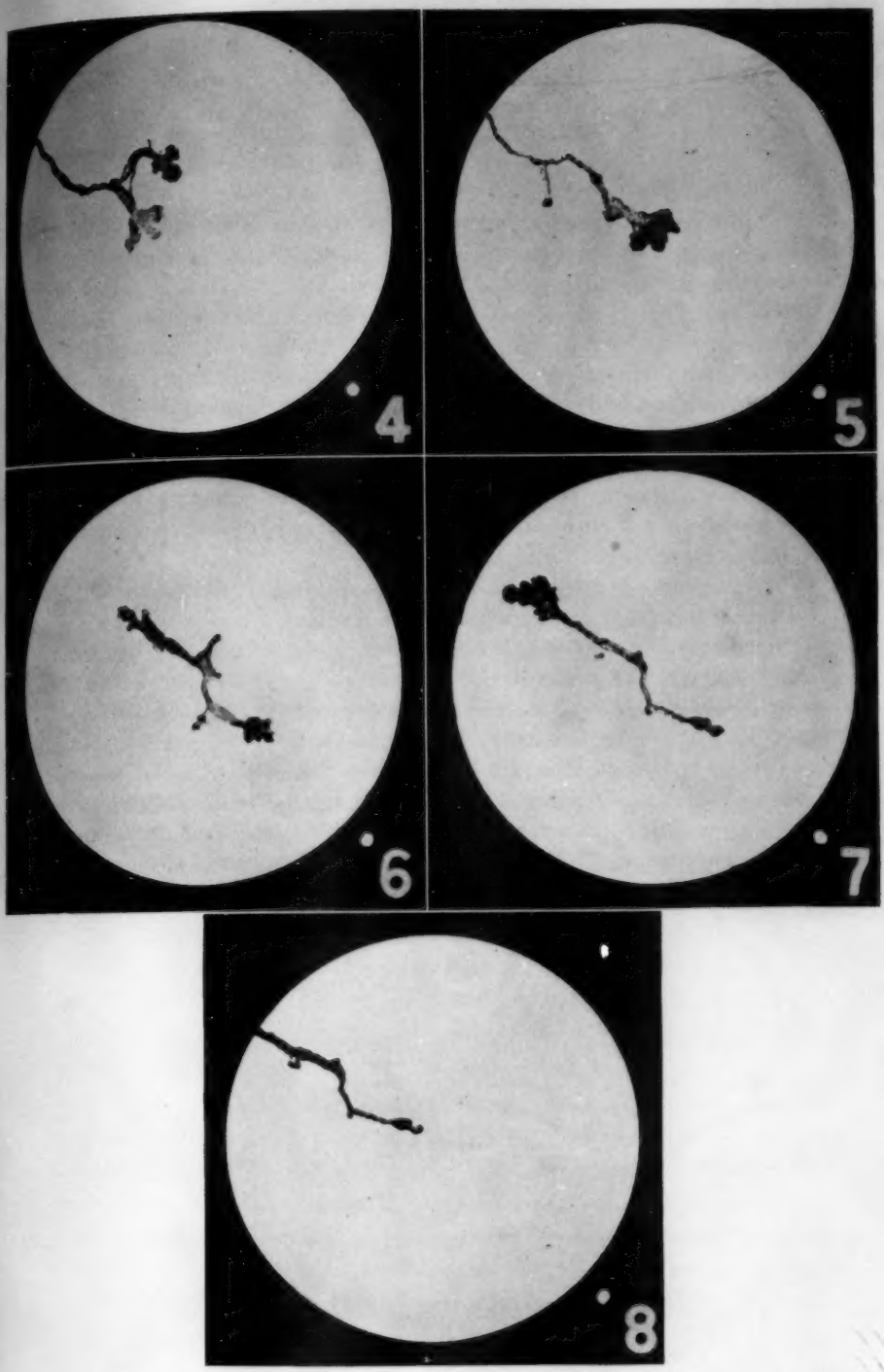
The positive and negative responses of this "cell" to chemical stimuli may be illustrated by a representative experiment. The appearance of the mold displaying positive chemotaxis is shown in figures 4 and 5. The source of attraction is just outside the photographic field and is indicated by the white dot. In figure 4 the organism is advancing into the microscopic field and shows two pseudopods at its anterior end; three minutes later, one of these pseudopods has retracted as the mold continues its advance. During all this time, of course, the rhythmic protoplasmic streaming goes on, being of longer duration in the direction of the attracting substance.

After the latter photograph was taken, the attracting substance (oatmeal) was removed, and a repelling substance, a silicate, was placed in the same position. Figures 6, 7 and 8 show the subsequent behavior of the organism. Within three minutes the advancing pseudopod seen in the preceding photograph has shrunk, and a new pseudopod has developed at the opposite end of the organism. In figure 7 the original pseudopod has nearly disappeared, while the pseudopod at the opposite end has broadened, and the organism has begun to move away from the repelling substance. In the last photograph (fig. 8) the mold is seen leaving the field.

Thus change from positive chemotaxis to negative has brought about reversal of polarity; the former "head" has become "tail," the former "tail" has become "head." The organism has not turned around but has "gone into reverse."

At the same time reorientation of the organism has occurred in other respects. It is now the end farthest from the negatively chemotactic substance that appears liquid, and at this end form clear blister-like extensions of the pseudopod, into which the granular protoplasm flows. At the end nearest the source of repulsion the changes are of opposite character. As this end contracts it becomes less fluid, i. e., protoplasmic inclusions move less turbulently, as if the substance were highly viscous. The duration of the phases of rhythmic streaming alters so that the flow is now longer in a direction away from the repelling substance.

Thus the organism when reacting negatively is the mirror image of itself when reacting positively. The difference is simply that between minus and plus; there is no qualitative difference. If the observer saw the organism but not the source of the substance to which it reacts, he would be unable to say whether chemotaxis was positive or negative, since the appearance of the organism and the rhythm of streaming are the same in both cases.



EXPLANATION OF FIGURES 4 TO 8

These are low power photomicrographs of a small piece of plasmodium, taken at intervals of three minutes, to show its appearance while it is displaying positive and negative chemotaxis, respectively.

In figures 4 and 5 the plasmodium is advancing toward the test substance (oatmeal) located outside the microscopic field at the position indicated by the white dot. After the latter photograph was taken the attracting substance, oatmeal, was removed, and a repelling substance, a silicate (Lloyd's reagent), was substituted. Figures 6, 7 and 8 show the plasmodium as it reverses its form and direction of movement, being repelled by the silicate.

SUMMARY

In order to supplement previous studies with leukocytes, the differences between positive and negative chemotaxis were investigated in a primitive free-living organism, Physarum polycephalum, a myxocyte. This organism, because of its large size, robustness and ease of handling, is better adapted to certain experiments than are leukocytes.

In gross preparations (visible to the naked eye) the substances tested included sugars, acids and alkalis. To dextrose chemotaxis was positive; to saccharose it was indifferent. Both hydrogen and hydroxyl ions in adequate concentrations induced negative chemotaxis, i. e., repelled the organism. Thus the positive or negative character of the reaction seems to depend on the chemical nature of the substance rather than merely on the concentration.

In microscopic preparations a small bit of the organism, comparable to a macrophage, was caused to alter its response from positive to negative. There was no qualitative difference in the behavior of the organism; when reacting negatively, it presented a mirror image of itself reacting positively. When altering its response, the organism does not turn around but "goes into reverse."

It cannot be stated that the chemotactic responses of the myxomycete and leukocyte are identical, but so similar are their mode of locomotion and chemotactic behavior that the information gained from the myxomycete should be helpful in further studies of negative chemotaxis in leukocytes.

INDUCED PULMONARY TUMORS IN MICE

1. SUSCEPTIBILITY OF SEVEN STRAINS OF MICE TO THE ACTION OF INTRAVENOUSLY INJECTED METHYLCHOLANTHRENE

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The primary pulmonary tumor, next to mammary carcinoma, is the most common type of neoplasm in mice. The various inbred strains of mice that have been established in laboratories throughout the world show marked differences in susceptibility to the occurrence of pulmonary tumors, and it has been asserted that the susceptibility is a genetic dominant characteristic.[1] The tumors can be induced by the introduction of carcinogenic hydrocarbons into the animals. The susceptibility to the induction of primary pulmonary tumors varies markedly in different strains of mice and is parallel to their susceptibility to the spontaneous occurrence of growths; i. e., the mice most susceptible to the spontaneous occurrence of tumors of the lungs are also most susceptible to the induction of pulmonary tumors with carcinogens.[2]

In 1938 Andervont [3] published a study on the comparative susceptibility of eight inbred strains of mice to the spontaneous occurrence, the induction and the transplantation of tumors. The susceptibility to induction of primary pulmonary tumors was determined from animals given 1, 2, 5, 6-dibenzanthracene subcutaneously.

This is a report of a similar investigation, comparing the susceptibility of seven inbred strains of mice to the induction of primary pulmonary tumors by intravenously injected 20-methylcholanthrene. The intravenous route of injection is more advantageous for this determination because the dose of the hydrocarbon coming in contact with the lungs is directly ascertainable and because tumors at the sites of subcutaneous injection are avoided. The study was undertaken to ascertain whether the use of a different carcinogenic hydrocarbon and a different

1. Bittner, J. J.: Pub. Health Rep. **53**:2197, 1938.
2. Andervont, H. B.: Pub. Health Rep. **54**:1512, 1939.
3. Andervont, H. B.: Pub. Health Rep. **53**:1647, 1938.

route of administration would influence the results and to compare the histologic appearance of the pulmonary tumors elicited in the various strains of mice.

EXPERIMENTAL PROCEDURE

The experiment was started in December 1938. Mice of strains C, I, Y, C_3H and C57 black were born and raised in this laboratory, and mice of strains A and L (or strain M [leaden]) were obtained from the Roscoe B. Jackson Memorial Laboratory. The animals were kept under identical environmental conditions. With the exception of the C57 blacks, a strain in which only males were available, equal numbers of males and females were used.

When 2 to 3 months of age, 38 A, 33 C, 27 I, 24 Y, 26 C_3H, 21 C57 black and 22 L strain mice were each given intravenously, in the lateral tail vein, an injection of 0.5 mg. of methylcholanthrene dispersed in 0.5 cc. of horse serum and cholesterol. The dispersion was prepared according to a technic described previously.[4]

Five or more animals of each strain were killed and examined six, thirteen, twenty, twenty-six and thirty-two weeks after the injection. The lungs were fixed in Tellyesniczky's fluid and were examined grossly for pulmonary nodules; nodules visible on the external surfaces of the lungs were counted. Single sections of the lungs were stained with hematoxylin and eosin.

RELATIVE SUSCEPTIBILITIES OF SEVEN STRAINS OF MICE

Five mice died of intercurrent infection in the course of the experiment, and data on these were not included in the study. At postmortem examination, none of the mice used in this study was observed in the gross to have tumors other than pulmonary tumors. In about half of the C57 black mice, however, chronic pneumonia developed, with large white patches of inflammatory reaction in the lungs.

Table 1, presenting the results, shows that mice of strain A are much more susceptible to the induction of pulmonary tumors by intravenous injection of methylcholanthrene than any of the other six strains. In six weeks all of the animals showed multiple tumors of the lungs. As a matter of fact, 0.5 mg. of methylcholanthrene introduced intravenously induced an average of 5 pulmonary tumors in 80 per cent of strain A mice four weeks after injection.[5] The number of tumors in each pair of lungs increased as the time after injection progresses. Thus, at six weeks each mouse had from 8 to 48 nodules, or an average

4. (*a*) Lorenz, E., and Andervont, H. B.: Am. J. Cancer **26**:783, 1936. (*b*) Andervont, H. B., and Lorenz, E.: Pub. Health Rep. **52**:637, 1937. The dispersion was prepared by Dr. Lorenz. The hydrocarbon was a synthetic product with a melting point of 179.8-180.4 C. (corrected), and the final concentration was checked by absorption spectrum analysis.

5. Shimkin, M. B.: Arch. Path., this issue, p. 239.

TABLE 1.—*Susceptibility of Seven Strains of Mice to a Single Intravenous Injection of 0.5 Mg. of Methylcholanthrene*

Strain of Mouse	Spontaneous Incidence of Tumors in Lungs of Mice over 1 Year Old, %	Susceptibility to Induction of Pulmonary Tumors by Subcutaneous Injection of Dibenzanthracene[3]	6 Weeks			13 Weeks			20 Weeks			26 Weeks			32 Weeks		
			Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice	Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice	Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice	Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice	Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice
A	70-85	Very high	10	10	25	21	21	30	6	6	47	..	..	..	..	..	..
C	20-30	High	2	0	0	5	0	0	6	6	4	6	6	13	14	14	40
I	10-20	Medium	..	..	..	5	0	0	6	2	3	16	12	3	..	..	..
Y	10-20	Medium	3	0	0	5	1	1	5	2	3	10	6	3	..	..	..
CsH	5-10	Medium	3	0	0	5	0	0	5	0	0	13	6	2	..	..	..
C57 black.....	0-1	Low	..	..	..	5	0	0	7	0	0	6	1	1	..	..	..
L	.....	Low	..	..	..	7	0	0	5	0	0	5	0	0	5	1	2

of 25 nodules per animal, and at twenty weeks the average number of pulmonary tumors per strain A mouse was 47. No pulmonary tumors were seen in the other strains at six weeks, nor at thirteen weeks except for a single pulmonary nodule in a strain Y mouse.

Strain C is next in order of susceptibility to development of tumors of the lung, which appeared in animals killed twenty weeks after injection of the hydrocarbon. An increase in the number of pulmonary tumors per animal as the time after injection increased was also evident in this strain.

Strains I and Y are of medium susceptibility; the I mice are slightly more susceptible than the Y animals. Tumors began to occur in mice killed at twenty weeks. Six weeks later about 60 per cent of the animals showed nodules, and the affected mice had an average of about 3 nodules per pair of lungs. The C_3H strain is less susceptible than the I or Y strains; tumors were observed in half of the animals twenty-six weeks after injection.

Strains C57 black and L are comparatively resistant to the induction tumors of the lung. One tumor appeared in a C57 black mouse in twenty-six weeks, and two tumors in a mouse of the L strain in thirty-two weeks.

Iball [6] proposed that the relative potency of carcinogenic compounds can be expressed by the index $\frac{P}{T} \times 100$, in which P is the percentage of animals in which tumors develop and T the average latent period in days. A similar index can be devised for the relative susceptibility of various strains of mice to pulmonary tumors: $\frac{P \times N}{T} \times 100$, in which P is the percentage of animals in which tumors of the lungs develop, N the average number of pulmonary tumors per tumor-bearing animal, and T the *minimal* latent period for the appearance of tumors of the lungs, in days. According to this index, the relative susceptibilities of the seven strains of mice to 0.5 mg. of methylcholanthrene injected intravenously can be expressed as: strain A, 1,400; strain C, 300; strains I and Y, 100; strain C_3H, 50, and strains C57 black and L, 10.

PATHOLOGIC OBSERVATIONS

Primary pulmonary tumors in mice have been described on numerous occasions, and Tyzzer's report [7] is still unexcelled. Recently, Grady and Stewart,[8] of this laboratory, studied the histogenesis of the induced pulmonary tumors in strain A mice; the evidence is that at least the great

6. Iball, J.: Am. J. Cancer **35**:188, 1939.

7. Tyzzer, E. E.: J. M. Research **21**:479, 1909.

8. Grady, H. G., and Stewart, H. L.: Am. J. Path., to be published.

majority of these neoplasms arise from the cells of the alveoli and not from the bronchi. They are adenomatous tumors, extremely uniform in appearance. The nodules, situated usually just below the pleura and connected with the thickened pleura, consist of moderately large round or cuboid cells with definite boundaries and large round or oval nuclei. The growth is fairly solid, and in many areas the cells are arranged around a central cavity, suggesting alveolar arrangement. Blood vessels are few, and mitoses are rare. The tumors are not encapsulated.

The induced tumors, after appearing rather suddenly, grow slowly, so that in six months the nodules may progress from pinpoint size to a diameter of a few millimeters. Many of the older tumors have the same appearance as the younger ones, but some show morphologic changes; the cells become larger, the tumor is more glandular and papillary, and occasional cystic areas are encountered. The larger tumors may lie in contact with bronchioles, but actual invasion of these structures by the tumor is not common.

It is believed that practically all these tumors are identical, and they are therefore referred to simply as "adenomatous tumors."

Slye, Holmes and Wells,[9] in a study of 160 spontaneous pulmonary tumors in mice, classified 20 as "unquestionably carcinomas," 43 as showing "a reasonably sure malignant tendency," 41 as of "doubtful malignancy" and 56 as "benign." It is possible that the neoplasms originate as nonmalignant tumors and then change more or less rapidly into the malignant type.[10] That all these tumors either originally or eventually are malignant is suggested by the following: (1) They are not encapsulated and they invade the lung tissue; (2) there is progressive growth, although actual death of the animal cannot be attributed to them; (3) they change in morphologic nature with age; (4) they are transplantable to mice of the same strain, and in the subcutaneous tissues often assume a sarcomatous appearance,[11] and (5) metastases outside the lungs, although rare, have been demonstrated.[12]

In this study, only single sections of the lungs were taken; a final decision concerning many points has to await observations on serial sections. One hundred and twenty-six individual induced primary pulmonary tumors, occurring in 78 mice, were gathered, some from the pathologic collection at this laboratory, so that tumors induced in various

9. Slye, M.; Holmes, H. F., and Wells, H. G.: J. M. Research **30**:417, 1914.
10. Campbell, J. A.: Brit. J. Exper. Path. **18**:215, 1937.
11. Andervont, H. B.: (*a*) Pub. Health Rep. **52**:347, 1937; (*b*) **54**:1519, 1939.
12. Slye and others.[9] Campbell.[10]

ways and by dibenzanthracene as well as by methylcholanthrene were included. Twelve spontaneous single tumors were also considered (table 2).

All of the pulmonary tumors, with the questionable exception of 2 tumors in C_3H female mice in which the possibility of metastases from tumor of the breast could not be excluded, were remarkably uniform in appearance (figs. 1 and 2). A few older tumors, which had been present over seven months, looked slightly different; of these, 4 had a cystadenomatous structure, and 2 contained spindle cells as well as the adenomatous cells. Of the 138 tumors, 111 were directly subpleural, and thickening of the pleura was noted with 84. Nineteen of the tumors, 12 of which were seven months old or older, were noted to have mitoses; more than one mitotic figure, up to eight, were present in only 5 tumors,

TABLE 2.—*Pulmonary Tumors in Mice Examined Histologically*

Strain	Mice	Number Induced Tumors of Lungs	Number Spontaneous Tumors of Lungs	Number Subpleural Tumors	Number Tumors with Thickened Pleura	Number Tumors Showing Mitoses
A	41	67	6	57	43	5
C	16	30	..	27	20	8
I	10	8	2	7	3	1
Y	9	7	3	8	6	2
C^3H	8	8	1	7	7	3
C57	5	5	..	4	4	0
L	1	1	..	1	1	0
Total	90	126	12	111	84	19

all from mice killed at least six months after the introduction of the carcinogen.

There was no difference of appearance (except that due to age) between (1) spontaneous and induced tumors; (2) tumors of the six strains of inbred mice; (3) tumors produced by methylcholanthrene and tumors produced by dibenzanthracene; (4) tumors produced by different routes of injection (subcutaneous, intravenous or intratracheal [13]); (5) tumors induced with the carcinogen in different physical states (dissolved in lard, dispersed in horse serum, as cholesterol pellet, or adsorbed on charcoal). Andervont [14] reported that pulmonary tumors produced in strain A mice with 2-amino-5-azotoluene or 3,4,5,6-dibenzcarbazole are also similar macroscopically and microscopically to tumors induced by the hydrocarbons.

13. Shimkin, M. B.: Am. J. Cancer **36**:538, 1939.

14. Andervont, H. B.: Pub. Health Rep. **54**:1529, 1939.

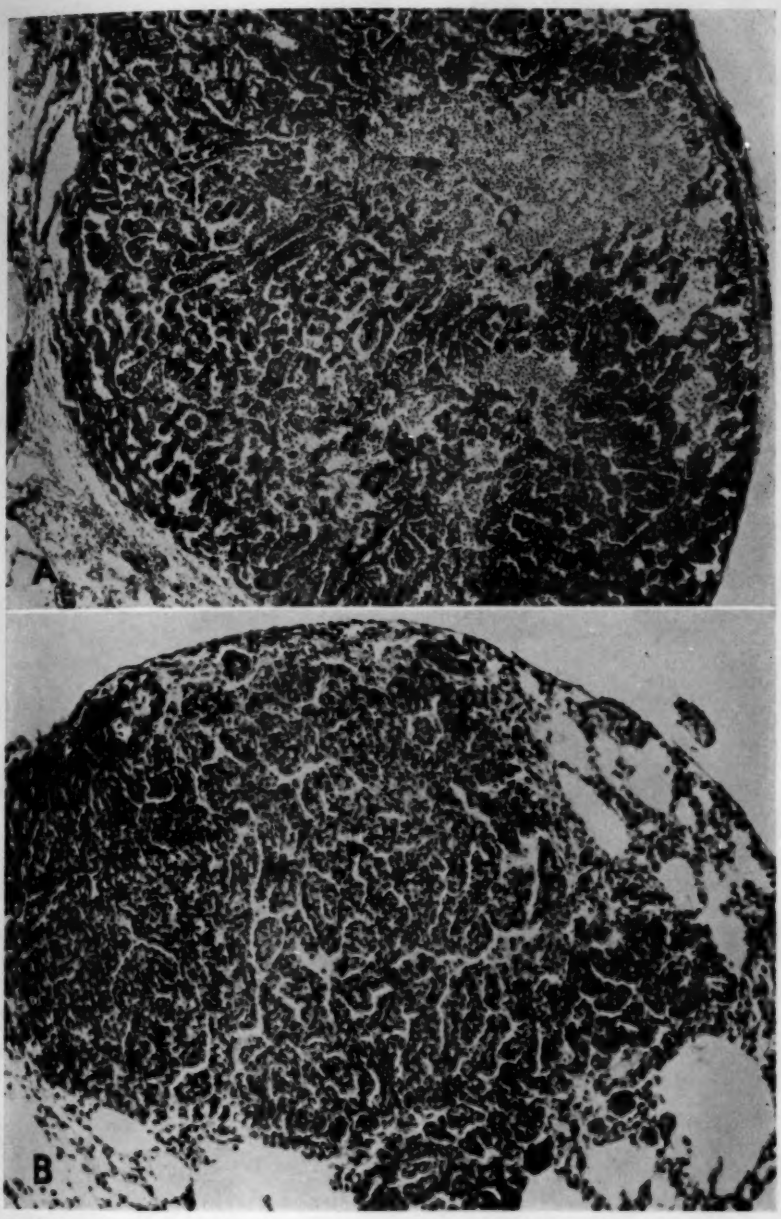

Fig. 1.—*A*, spontaneous pulmonary tumor in a male of strain C$_3$H 15 months old; hematoxylin and eosin; × 50. *B*, induced pulmonary tumor in a male of strain C57 black twelve months after subcutaneous injection of 0.8 mg. of 1,2,5,6-dibenzanthracene in lard; hematoxylin and eosin; × 100.

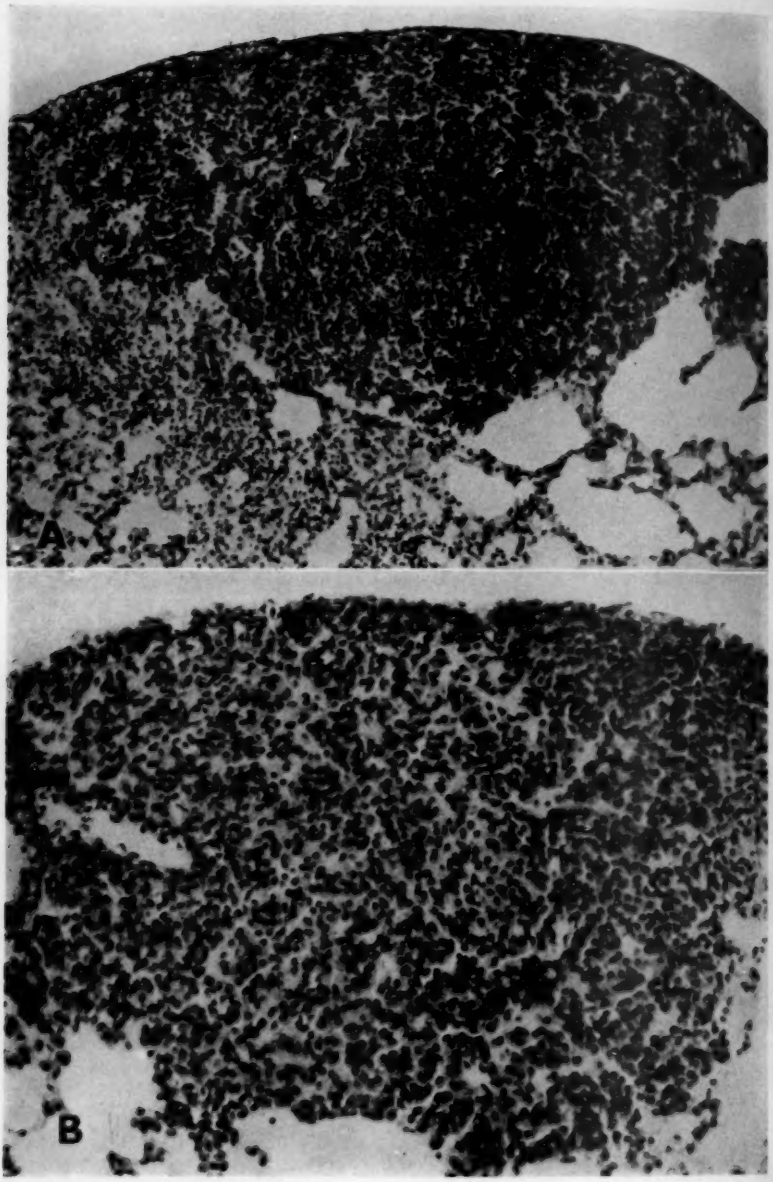

Fig. 2.—*A*, induced pulmonary tumor in a male of strain Y five months after subcutaneous injection of 2 mg. of methylcholanthrene in lard; hematoxylin and eosin; × 100. *B*, induced pulmonary tumor in a female of strain A, three months after intravenous injection of 0.5 mg. of methylcholanthrene in horse serum; hematoxylin and eosin; × 200.

COMMENT

The relative susceptibilities of seven strains of mice to induction of primary pulmonary tumors by intravenously injected methylcholanthrene agree with the results obtained by Andervont [3] with subcutaneously injected 1,2,5,6-dibenzanthracene in lard. He determined that strain A mice were highly susceptible to the induction of pulmonary tumors by this agent and that the C strain was next in susceptibility; strains I, C_3H and Y were designated as of medium susceptibility, and strains C57 black, L and D as resistant. Tumors of the lungs were induced in all strains, however, including the three resistant ones.[15]

The use of methylcholanthrene rather than 1,2,5,6-dibenzanthracene, and the use of the intravenous route of injection instead of the subcutaneous, therefore, did not influence the results.

It is also evident that the susceptibility of the strains to induction of pulmonary tumors is parallel to their susceptibility to spontaneous development of pulmonary tumors. Thus, over 70 per cent of strain A mice over a year old have tumors of the lungs [1]; even at six months the incidence is about 10 per cent.[16] The great majority of the spontaneous tumors in strain A mice, as in the other strains, are single, in contrast with the multiple induced tumors.

In strain C mice over a year old the incidence of spontaneous tumors of the lungs is 20 to 30 per cent.[3] Recent data obtained by Andervont [16] show that in 187 strain I mice of an average age of 15 months 30 pulmonary tumors were seen (16 per cent) and that of 110 strain Y mice of an average age of 13 months, 11 (10 per cent) had pulmonary tumors. Of 165 C_3H mice about 14 months old, 11 (7 per cent) had tumors of the lungs.[17] The reported incidence of spontaneous pulmonary tumors in strain C57 black over a year old is less than 1 per cent.[18] The incidence of pulmonary tumors in the L mice has not been recorded, but the strain is related to the C57 and is resistant to spontaneous tumors of the lungs.[15]

The parallelism between the susceptibility to spontaneous development of pulmonary tumors and that to induction of pulmonary tumors with carcinogenic hydrocarbons suggests that the hydrocarbons are accelerators of some process inherent in the animals. It is interesting that the estrogens have a similar action; male mice of strains in which the females are highly susceptible to spontaneous development of mammary cancer readily acquire mammary tumors on treatment with estrogens, while male

15. Andervont, H. B.: Pub. Health Rep. **54**:1524, 1939.

16. Andervont, H. B.: Unpublished data.

17. Andervont, H. B.: Pub. Health Rep. **54**:1158, 1939.

18. Little, C. C.; Murray, W. S., and Cloudman, A. M.: Am. J. Cancer **36**: 431, 1939.

mice of strains in which the females have a low incidence of this type of tumor are much more resistant to the agent.[19] However, with sufficient carcinogen or estrogen and sufficiently long periods of time, pulmonary tumors can be elicited with the former and mammary tumors with the latter even in the resistant strains of animals.[20] The difference in susceptibility of various strains of mice to induction of pulmonary tumors is apparently a matter of degree, and, as Andervont stated,[2] hereditary factors exert their influence by controlling the degree of susceptibility.

SUMMARY

Seven strains of mice were tested for their susceptibility to induction of primary pulmonary tumors by the intravenous injection of 20-methylcholanthrene. Strain A is most susceptible, strain C is next in susceptibility, strains Y, I and C_3H are of medium susceptibility, and strains C57 black and L are relatively resistant.

The relative susceptibility to induction of primary pulmonary tumors is parallel to the susceptibility to spontaneous development of pulmonary tumors; i. e., mice which show the greatest number of induced pulmonary tumors also show the greatest number of spontaneous tumors of the lungs.

The pulmonary tumors induced in mice are adenomatous and almost identical in appearance. There is no morphologic difference (1) between spontaneous and induced tumors; (2) between tumors induced in the seven strains of mice; (3) between tumors produced by methylcholanthrene and those produced by 1,2,5,6-dibenzanthracene; (4) between tumors induced by different routes of injection (subcutaneous, intravenous and intratracheal) or (5) between tumors induced by the carcinogen in different states (in lard, in horse serum, as cholesterol pellet or adsorbed on charcoal).

19. Gardner, W. U.: Arch. Path. **27**:138, 1939.

20. Twombly, G. H.: Proc. Soc. Exper. Biol. & Med. **40**:430, 1939.

INDUCED PULMONARY TUMORS IN MICE

II. REACTION OF LUNGS OF STRAIN A MICE TO CARCINOGENIC HYDROCARBONS

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The inbred strain of albino mice designated as strain A was established by Strong,[1] in 1921. The breeding females have a high incidence of mammary carcinoma, and both males and females are very susceptible to the development of primary pulmonary tumors[2]; the occurrence of other types of neoplasm in the stock has also been recorded.[3]

The lungs of strain A mice are extremely susceptible to the induction of tumors with carcinogenic agents and are a desirable tissue for many phases of investigation of the action of cancer-provoking compounds. This paper describes the response of the lungs of strain A mice to three carcinogenic polynuclear aromatic hydrocarbons, with special reference to the effects of dose, of the route of injection, of the medium for the compounds and of the time after injection of the appearance of the induced primary pulmonary tumors. The studies are based on previous work done in this laboratory by Dr. H. B. Andervont[4] and were initiated in December 1938.

EXPERIMENTAL PROCEDURE

The inbred strain A mice used in these investigations were obtained from the Roscoe B. Jackson Memorial Laboratory, of Bar Harbor, Maine. They were maintained on Purina dog chow,[5] with an unlimited supply of water, and under

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From the National Cancer Institute, United States Public Health Service.

1. Strong, L. C.: J. Hered. **27**:21, 1936.

2. Bittner, J. J.: (*a*) Am. J. Cancer **27**:519, 1936; (*b*) Pub. Health Rep. **53**:2197, 1938; **54**:380, 1939.

3. Cloudman, A. M.; Bittner, J. J., and Little, C. C., in Some Fundamental Aspects of the Cancer Problem, Symposium Sponsored by the Section on Medical Sciences of the American Association for the Advancement of Science, New York, The Science Press, 1937, p. 37.

4. Andervont, H. B.: (*a*) Pub. Health Rep. **52**:212, (*b*) 304, (*c*) 347 and (*d*) 1584, 1937; (*e*) **53**:229, 1938; (*f*) **54**:1512, (*g*) 1519, (*h*) 1524 and (*i*) 1529, 1939.

5. According to the manufacturer, the chow contains the following ingredients: protein, 20 per cent; fat, 3 per cent; carbohydrate, 56 per cent; ash, 6 per cent, and water, 15 per cent, with vitamins A and G added.

identical environmental conditions. They were from 2 to 3 months of age at the start of the experiments, and the experimental groups usually included equal numbers of males and females, which were kept separate.

The carcinogens employed were 20-methylcholanthrene, 1,2,5,6-dibenzanthracene and 3,4-benzpyrene.[6] The compounds were purified by Dr. J. L. Hartwell, and the melting points were 179.8-180.4 C., 266.0-266.8 C. and 178.6-179.0 C. (corrected), respectively. The dispersions of methylcholanthrene and dibenzanthracene in horse serum and cholesterol were prepared by Dr. Egon Lorenz according to a technic described previously,[7] and the final concentrations were checked by absorption spectrum analysis.

At designated periods after the injection of the carcinogens the animals were killed by cervical dislocation and autopsies made grossly. The lungs were examined for the presence and the number of white nodules and were fixed in Tellyesniczky's fluid. After fixation the nodules in the lungs, especially when small, became more evident; those in each pair of lungs were counted and the number recorded. The tissue was sectioned and stained with hematoxylin and eosin for histologic verification.

RESULTS

Since the great majority of pulmonary tumors in mice is found directly underneath the pleura,[8] it was possible to count the tumors on the external surfaces of the lungs with gratifying accuracy, especially after fixation. Histologic sections attested that the gross appearance of the pulmonary tumors was characteristic: they were pearly, shiny, round areas, slightly raised and sharply distinct from the surrounding lung tissue (fig. 1). Occasional errors in the gross diagnosis were encountered in mice with chronic pneumonic infiltration of the lungs or with large lymphatic patches; in most of these, however, differentiation was easy, as the inflammatory or lymphomatous patches appeared as extensive, silghtly nodular or smooth irregular grayish areas with ill defined boundaries.

The induced pulmonary tumors in strain A mice (and in other inbred strains as well) are morphologically identical with the pulmonary tumors of spontaneous origin except for their frequency and multiplicity.[8] They arise from the alveolar lining of the lung[9] and occur at the same site, just beneath the pleura. They are of the same macroscopic and microscopic appearance: adenomatous tumors which with advancing age often assume a papillary structure. Recognizable grossly when they are of pinpoint size, the tumors grow rapidly to a few millimeters in diameter, and then remain fairly stationary (figs.

6. Throughout the paper the compounds are referred to as methylcholanthrene, dibenzanthracene and benzpyrene.

7. (*a*) Lorenz, E., and Andervont, H. B.: Am. J. Cancer **26**:783, 1936. (*b*) Andervont, H. B., and Lorenz, E.: Pub. Health Rep. **52**:637, 1937.

8. Shimkin, M. B.: Arch. Path., this issue, p. 229.

9. Grady, H. G., and Stewart, H. L.: Am. J. Path., to be published.

1 and 2); death of the animal is not attributable to their presence. Inflammatory reaction is significantly absent in and around the tumors.[9] The site and the morphologic character of the induced pulmonary tumors are not altered by variations in the carcinogen, the medium of the carcinogen or the route of injection.[8]

Both the spontaneous and the induced pulmonary tumors are transplantable in the subcutaneous tissue of mice of the same strain, where they often undergo a remarkable change in appearance and become spindle cell tumors.[4e, g]

That these pulmonary tumors are originally or eventually malignant is suggested by their invasion of the lung and lack of encapsulation,

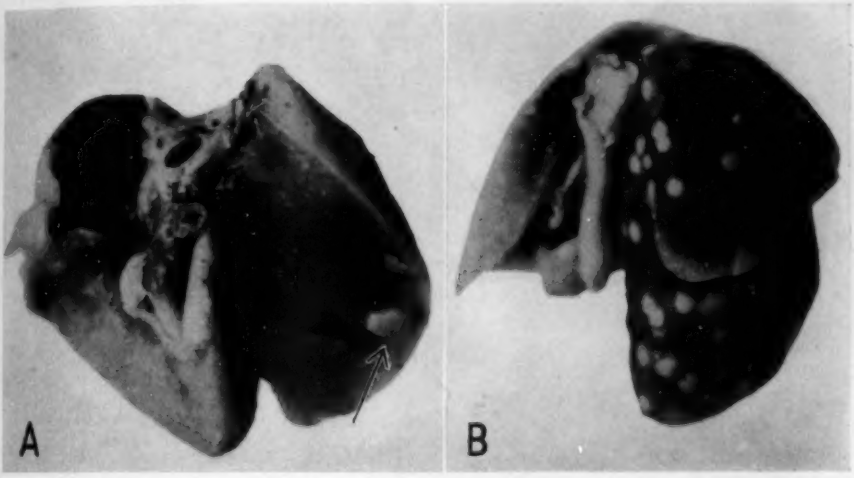

Fig. 1.—*A*, spontaneous pulmonary tumor in strain A male mouse 8 months old; × 3.5. *B*, induced multiple pulmonary tumors in strain A male mouse thirteen weeks after an intravenous injection of 1.5 mg. of methylcholanthrene dispersed in 1.5 cc. of horse serum and cholesterol; × 3.5.

their progressive growth and change in appearance, their transplantability and the demonstration of occasional metastases outside the lung.[10]

Experiment 1. Incidence of Spontaneous Lung Tumors.—Knowledge of the incidence of spontaneous pulmonary tumors in strain A mice is an obvious essential for the interpretation of results with carcinogenic agents. Bittner [2] reported the incidence of pulmonary tumors in strain A mice of advanced age; at an average age of 14.8 months, 71.6 per cent have pulmonary tumors, and the incidence rises to 89.2 per cent at an average age of 19.5 months. This information, compiled according to the average age rather than month by month, does not meet the

10. (*a*) Slye, M.; Holmes, H. F., and Wells, H. G.: J. M. Research **30**:417, 1914. (*b*) Campbell, J. A.: Brit. J. Exper. Path. **15**:287, 1934; **18**:215, 1937.

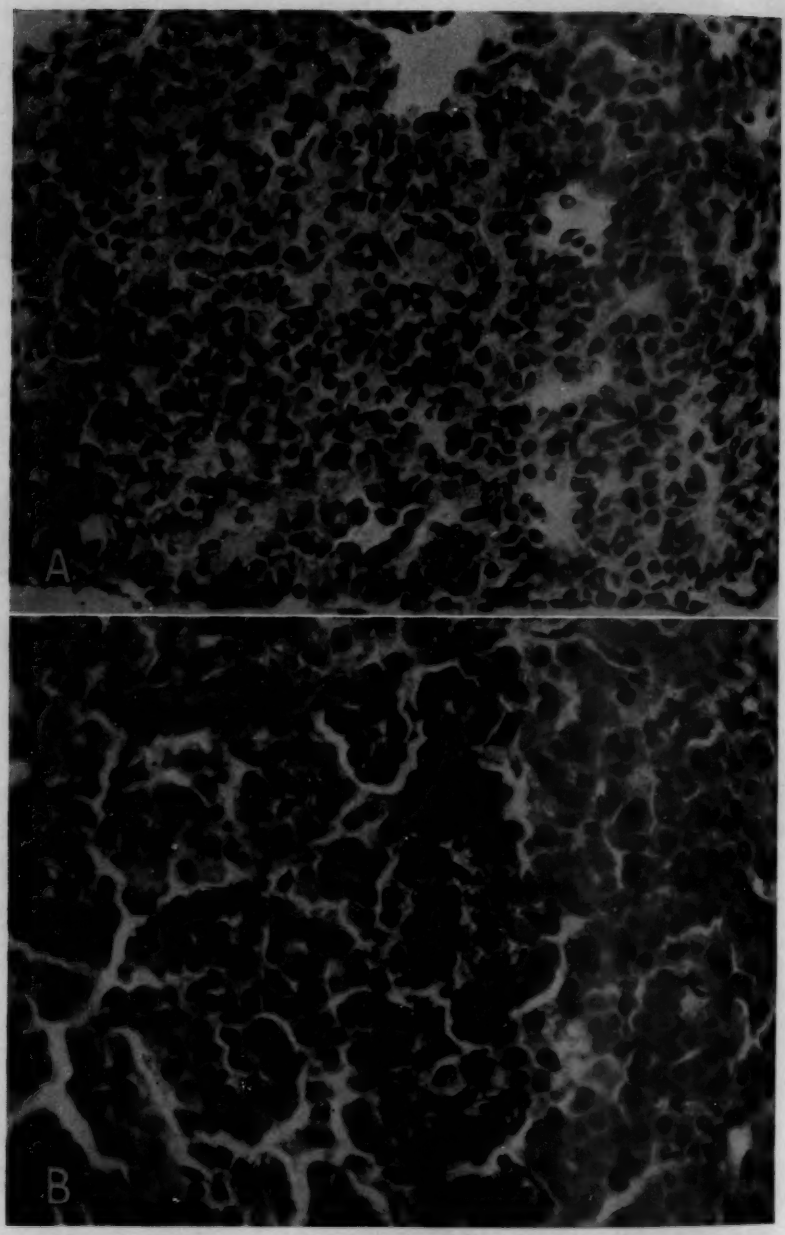

Fig. 2.—*A*, spontaneous pulmonary tumor in strain A male mouse 10 months old; hematoxylin and eosin; × 200. *B*, induced pulmonary tumor in strain A female mouse six weeks after intravenous injection of 0.5 mg. of methylcholanthrene dispersed in 0.5 cc. of horse serum and cholesterol; hematoxylin and eosin; × 400.

requirement of experiments with carcinogens, which often are terminated long before the animals reach such ages.

The data on the incidence of spontaneous pulmonary tumors in strain A mice under 1 year of age were therefore gathered and supplemented by those from additional autopsies. The mice included both males and females, as sex does not influence the incidence of these tumors.[2b]

Table 1 shows that pulmonary tumors appear in strain A mice as young as 3 months, and that the incidence rises gradually toward the figures given by Bittner. At 8 or 9 months of age, when the longest experiments described here were terminated, the incidence is 17 to 35 per cent.

Of special interest is the fact that few of the spontaneous pulmonary tumors in strain A mice are multiple. Thus, up to 10 months of age only 1 animal with more than 1 pulmonary tumor was found in a total of 43 mice bearing tumors of the lungs. The incidence of multiple

TABLE 1.—*Experiment 1. Incidence of Spontaneous Pulmonary Tumors in Strain A Mice Under 1 Year Old*

Age, Mo.	Mice	Number with Tumors of the Lungs	Percentage with Tumors of the Lungs	Number with Multiple Tumors of the Lungs
3	40	1	2.5	0
4	25	1	4.0	0
5	29	2	6.8	0
6	43	4	9.3	0
7	75	11	14.7	0
8	52	9	17.5	1
9	43	15	34.5	0
10	45	21	42.2	3

tumors increases with advance in age; of 31 pulmonary tumors seen in mice over a year old (average age, 15 months), 7 were multiple. Usually these mice have 2 tumors per pair of lungs, but up to 10 tumors in an animal 16 months of age have been observed. In judging whether pulmonary tumors in mice are induced by any given agent or are of spontaneous origin, the number of pulmonary tumors per animal as well as the incidence of such tumors in a group of animals must be taken into consideration.

Experiment 2. Intravenous Injection of Methylcholanthrene.—Strain A mice 2 months of age, with an equal number of males and females in each group, were given intravenously, in a lateral tail vein, methylcholanthrene dispersed in horse serum and cholesterol to equal 1 mg. of the hydrocarbon per cubic centimeter of the serum, as follows:

(*a*) Forty animals received an injection of 0.5 cc., the amount tolerated without mortality. The dose was repeated in seven hours and again in sixteen hours, so that within twenty-four hours the mice received 1.5 mg. of methylcholanthrene in 1.5 cc. of horse serum. Eight mice died during the second and third injections.

(*b*) Sixty-four mice received a single injection of 0.5 mg. of methylcholanthrene in 0.5 cc. of horse serum. Two animals died of intercurrent infection before the termination of the experiment. The results in this group have been reported in part elsewhere.[8]

(*c*) Thirty-three mice were given 0.1 mg. of methylcholanthrene dispersed in 0.1 cc. of horse serum. This dose was found previously to cause pulmonary tumors in strain A mice within five months.[11]

The animals were killed at three, four, five, six, thirteen and twenty weeks after the injection of the carcinogen, and the incidence of pulmonary tumors in the group and the number of tumors per pair of involved lungs determined.

The results are summarized in table 2 and are recapitulated in graphic form in figure 3. Pinpoint tumors began to appear in the lungs within four weeks after the intravenous injection of 1.5 or 0.5 mg. of methylcholanthrene. The number of tumors and the size of the individual tumors increased with progress of time after the injection, so

TABLE 2.—*Experiment 2. Incidence of Pulmonary Tumors in Strain A Mice After Intravenous Injection of Methylcholanthrene*

	1.5 Mg.			0.5 Mg.			0.1 Mg.		
Time, Weeks	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs
3	6	1	1	9	1	1	..	..	..
4	7	7	12	10	8	5	..	..	..
5	10	10	22	10	10	14	..	..	..
6	4	4	55	10	10	25	7	3	2
13	5	5	74	18	18	30	10	8	4
20	..	..	..	6	6	47	15	15	11

that in three months the mice that received the larger dose had an average of 74 separate pulmonary tumors per pair of lung (fig. 1*B*).

It is to be noted that the average number of pulmonary tumors per animal, as well as the number of mice bearing tumors, is directly proportional to the dose of the hydrocarbon administered. Thus, at four weeks all mice given 1.5 mg. had tumors of the lungs, whereas 2 of 10 mice receiving 0.5 mg. were uninvolved. At six weeks all of the animals receiving either dose had multiple tumors of the lungs, but with 1.5 mg. the average number per mouse was 55, compared with 25 for animals receiving 0.5 mg. At this time, only half of the mice which had been given 0.1 mg. had pulmonary tumors, and the tumor-bearing animals had an average of 2 such tumors.

It has been proposed[8] that the relative susceptibilities of various inbred strains of mice to induction of pulmonary tumors by carcinogenic hydrocarbons can be expressed by the index $\frac{P \times N}{T} \times 100$, in which

11. Shimkin, M. B.: Am. J. Cancer **36**:538, 1939.

P is the percentage of animals in which pulmonary tumors develop, N the average number of such tumors per tumor-bearing animal, and T the minimal time in days in which the tumors appear.

In this experiment the validity of the index can be determined by the values obtained with the three doses of methylcholanthrene. For 1.5 mg., the index is 4,200; for 0.5 mg., 1,400, and for 0.1 mg., 250. The values are almost exactly proportional to the dose: 4,200: 1,400: 250 : : 1.5 : 0.5 : 0.1.

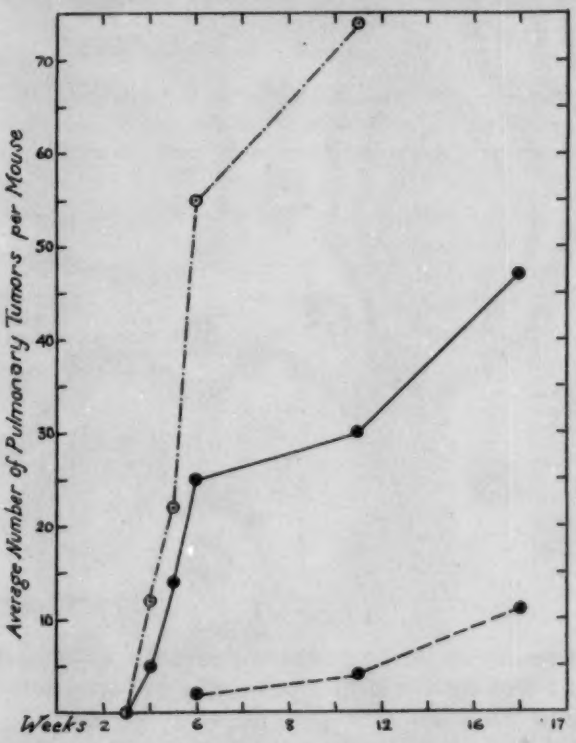

Fig. 3.—Response of the lungs of strain A mice to intravenously injected methylcholanthrene dispersed in horse serum. The dash line represents the response to 0.1 mg.; the solid line, that to 0.5 mg. and the dot-dash line that to 1.5 mg. (from table 2).

Experiment 3. Minimal Dose of Methylcholanthrene.—In order to ascertain the minimal intravenous dose of methylcholanthrene that would produce tumors of the lungs, groups of strain A mice 3 months old were given a single intravenous injection of the following dispersions of the compound in horse serum and cholesterol:

(*a*) 0.1 mg. of methylcholanthrene in 0.25 cc. of horse serum (10 mice)

(*b*) 0.05 mg. of methylcholanthrene in 0.25 cc. of horse serum (10 mice)

(*c*) 0.01 mg. of methylcholanthrene in 0.25 cc. of horse serum (20 mice)

(*d*) 0.001 mg. of methylcholanthrene in 0.25 cc. of horse serum (20 mice)

The animals given 0.1 mg. of methylcholanthrene in 0.1 cc. of horse serum (experiment 2) were included for comparison, and mice untreated or given injections of horse serum and cholesterol served as controls.

The mice were killed three to six months later, except for those given 0.001 mg. and half of the controls, which were kept for another month, when they were 10 months old.

As presented in table 3, 0.05 mg. of methylcholanthrene given intravenously induced tumors of the lungs, in 90 per cent of the mice within three months.

TABLE 3.—*Experiment 3. Minimal Dose of Methylcholanthrene Intravenously Required to Produce Tumors of the Lungs in Strain A Mice*

Dose of Methylcholanthrene, Mg.	Vehicle	Vol., Cc.	Mice Used	Time After Injection, Mo.	Age of Mice at Death, Mo.	Number Without Tumors of the Lungs	Mice Showing Given Number of Tumors per Mouse						Percentage of Mice with Tumors of the Lungs	Average Number of Tumors per Mouse
							1	2	3-5	6-10	11-20	20+		
0.1	Horse serum	0.25	10	3	6	2	..	2	3	3	..	..	80	4
0.1	Horse serum	0.1	15	4.5	7.5	0	..	3	2	6	4	2	100	11
0.05	Horse serum	0.25	10	3	6	1	5	3	..	1	..	..	90	2
0.01	Horse serum	0.25	5	3	6	4	1	..	..	..	..	..	20	1
0.01	Horse serum	0.25	15	6	9	9	5	1	..	..	..	..	40	1
0.001	Horse serum	0.25	10	6	9	5	4	1	..	..	..	..	50	1
0.001	Horse serum	0.25	10	7	10	7	2	1	..	..	..	..	30	1
.....	Horse serum	0.25	10	6	9	9	1	..	..	..	..	..	10	1
.....	Horse serum	0.25	10	7	10	6	3	1	..	..	..	..	40	1
.....	.....	....	10	..	9	9	1	..	..	..	..	..	10	1
.....	.....	....	10	..	10	5	5	..	..	..	..	..	50	1

The interpretation of the results with smaller doses is difficult. A dose of 0.01 mg. did not induce pulmonary tumors in three months; in six months the incidence was 40 per cent. At this age, 9 months, 10 per cent of the control animals had tumors of the lungs; it seems that the hydrocarbon accelerated their appearance. However, the 30 per cent incidence among the animals used to establish the occurrence of spontaneous pulmonary tumors (table 1) negates the finding.

From the data obtained, it can be concluded that 0.05 mg. of methylcholanthrene given intravenously induced pulmonary tumors in strain A mice within three months, and that 0.01 mg. perhaps accelerated their appearance. Concerning doses below 0.05 mg., it is felt that definite information cannot be derived from this experiment but could be elicited with greater numbers of experimental animals killed periodically in three to six months after injection.

Experiment 4. Intravenous Injection of Dibenzanthracene.—It has been reported [4f] that tumors of the lungs appear as quickly after the intravenous injection of dibenzanthracene as after that of methylcholanthrene. Since the carcinogenic index, which takes into consideration the multiplicity of induced pulmonary tumors as well as their incidence in the group and time of appearance, allows a more exact comparison of carcinogenicity and has been shown to be valid for three doses of methylcholanthrene (experiment 2), the following experiment was undertaken to evaluate the comparative cancer-provoking power of dibenzanthracene and methylcholanthrene. Percutaneous application and subcutaneous injection of the two compounds have established that methylcholanthrene is considerably more carcinogenic than dibenzanthracene in the induction of carcinomas and sarcomas.[12]

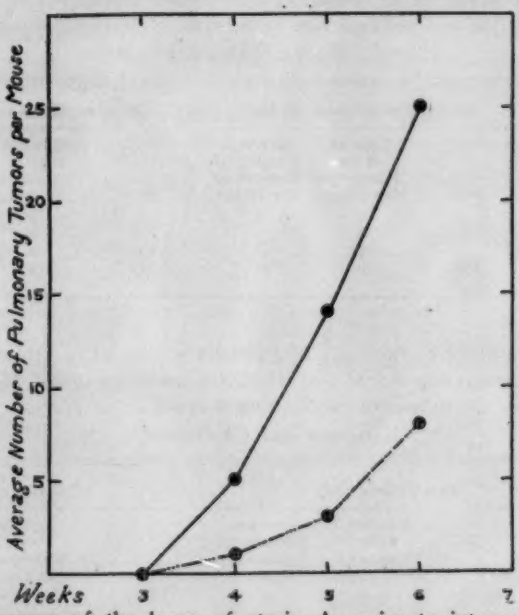

Fig. 4.—Response of the lungs of strain A mice to intravenously injected methylcholanthrene (solid line) and dibenzanthracene (broken line) dispersed in horse serum (from table 4).

Forty-one strain A mice 2½ months of age were given intravenously 0.5 mg. of dibenzanthracene dispersed in 0.5 cc. of horse serum and cholesterol. Ten animals were killed three, four, five and six weeks after injection and the lungs examined for the presence and the number of pulmonary tumors.

The results are given in table 4 and are compared with those obtained with the equivalent dose of methylcholanthrene; a graphic recapitulation is presented in figure 4. Tumors of the lungs appeared at approximately the same time as with methylcholanthrene, i. e., in four weeks. The incidence of pulmonary tumors in the group, as well as the average

12. (*a*) Iball, J.: Am. J. Cancer **36**:538, 1939. (*b*) Shimkin, M. B., and Andervont, H. B.: Pub. Health Rep., to be published.

number of tumors per animal, however, was significantly lower with dibenzanthracene. Thus, at four weeks 30 per cent had an average of 1 tumor per animal, as compared with 80 per cent with an average of 5 tumors with methylcholanthrene. At six weeks all animals that had received an injection of either hydrocarbon had tumors of the lungs, but the average number of tumors was significantly lower with dibenzanthracene.

The experiment indicates that dibenzanthracene is less carcinogenic than methylcholanthrene in inducing pulmonary tumors as well as in

TABLE 4.—*Experiment 4. Incidence of Pulmonary Tumors in Strain A Mice After Intravenous Injection of 0.5 Gm. of Methylcholanthrene or 0.5 Mg. of Dibenzanthracene*

	Methylcholanthrene, 0.5 Mg.			Dibenzanthracene, 0.5 Mg.		
Time, Weeks	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs
3	9	1	1	10	0	0
4	10	8	5	10	3	1
5	10	10	14	11	10	3
6	10	10	25	10	10	8

TABLE 5.—*Experiment 5. Incidence of Pulmonary Tumors in Strain A Mice Given Subcutaneously 0.5 Mg. of Methylcholanthrene or 0.5 Mg. of Dibenzanthracene Dispersed in 0.5 cc. of Horse Serum and Cholesterol*

	Methylcholanthrene			Dibenzanthracene		
Time, Weeks	Mice	Number with Tumors of the Lungs	Average Number of Pulmonary Tumors	Mice	Number with Tumors of the Lungs	Average Number of Pulmonary Tumors
3	10	0	0	10	0	0
4	10	2	1.0	7	0	0
5	10	5	1.6	6	2	1.0
6	10	7	2.2	7	5	2.0

inducing cutaneous carcinomas or subcutaneous sarcomas. It is suggested that the time of appearance, the incidence and the average number of pulmonary tumors per mouse in strain A mice after the intravenous introduction of a carcinogen (i. e., the carcinogenic index) can be used in determining quantitatively the relative carcinogenic power of the carcinogen in comparison with other compounds. The rapidity with which data are obtained, as compared with subcutaneous or percutaneous testing, is an additional advantage.

Experiment 5. Subcutaneous Injection of Methylcholanthrene or Dibenzanthracene Dispersion.—In order to ascertain the influence of the route of injection on the formation of pulmonary tumors, 40 strain A mice were given subcutaneously in the right axilla 0.5 mg. of methylcholanthrene dispersed in 0.5 cc. of horse serum

and cholesterol, and 30 strain A mice, the same amount of dibenzanthracene dispersion. The animals were killed at three, four, five and six weeks after the injection of the carcinogens and the lungs examined for the presence and the number of pulmonary nodules.

The results are presented in table 5. Pinpoint-sized tumors appeared in the lungs within four weeks after the injection of methylcholanthrene, and a week later with dibenzanthracene. The number of animals with pulmonary tumors and the average number of tumors per animal rose with progress of time after injection.

Although the slightly earlier appearance and the slightly greater average number of pulmonary tumors induced by methylcholanthrene

TABLE 6.—*Experiment 6. Incidence of Pulmonary Tumors in Strain A Mice Three Months After Subcutaneous Injection of Three Carcinogenic Hydrocarbons Dissolved in 0.25 cc. of Lard*

Hydrocarbon	Dose, Mg.	Mice Given Injection	Number Without Tumors of the Lungs	Mice with Given Number of Tumors per Mouse						Percentage of Mice with Tumors of the Lungs	Average Number of Tumors per Mouse	Carcinogenic Index
				1	2	3-5	6-10	11-20	20+			
Methylcholanthrene	0.25	10	3	3	2	2	..	..	..	70	1.8	125
Methylcholanthrene	0.5	10	2	2	2	4	..	..	..	80	2.5	200
Methylcholanthrene	1.0	11	1	1	3	4	..	2	..	91	4.3	390
Benzpyrene.....	0.25	10	6	3	..	1	..	..	..	40	1.5	60
Benzpyrene.....	0.5	9	6	3	..	..	..	..	..	33	1.0	35
Benzpyrene.....	1.0	10	5	2	2	1	..	..	..	50	2.2	110
Dibenzanthracene...	0.25	10	3	4	2	1	..	..	..	70	1.8	125
Dibenzanthracene...	0.5	10	0	3	1	5	1	..	..	100	2.8	280
Dibenzanthracene...	1.0	10	0	1	1	5	2	1	..	100	4.4	440

suggest the greater carcinogenicity of the agent as compared with dibenzanthracene, the difference that is so evident with the intravenous route of administration is obscured. It is apparent that the subcutaneous route of injection is not satisfactory for the more exact determination of relative carcinogenicities. By the intravenous route the compounds come in immediate and direct contact with the pulmonary tissues; when the injections are made subcutaneously, the production of tumors of the lungs is modified not only by the relative carcinogenicity of the compounds but probably primarily by the ability of the agents to dissolve and to reach the lungs. Thus, subcutaneously injected chemicals which are slowly absorbed from the site of injection and which are rapidly destroyed or eliminated would have little opportunity to induce pulmonary tumors, whereas with intravenous injection these factors would be minimized.

Experiment 6. Subcutaneous Injection of Methylcholanthrene, Dibenzanthracene or Benzpyrene in Lard.—Andervont [40] reported that lard solutions of

dibenzanthracene are not as efficacious as serum dispersions in producing pulmonary tumors in mice, probably because the carcinogen is maintained more firmly at the site of injection. The following experiment was made to determine the influence of lard solution as compared with that of serum dispersion, as shown in experiment 5, and to study the effect of dosage on the incidence of tumors in the group and the average number of pulmonary tumors per mouse.

Groups of 10 strain A mice 2 months of age were given subcutaneously in the right axilla 0.25, 0.5 or 1.0 mg. of methylcholanthrene, dibenzanthracene or benzpyrene dissolved in 0.25 cc. of best grade lard filtered at 37 C. Three months later all the animals were killed and the lungs examined for tumors.

The results are summarized in table 6. Tumors of the lungs were induced in all groups of mice within three months. In contrast with the observations with the intravenous route of administration (experiments 2 and 4) and possibly with the subcutaneous injection of the horse serum dispersion (experiment 5), dibenzanthracene in lard injected subcutaneously produced pulmonary tumors in a greater percentage of mice, as well as a greater number of pulmonary tumors per animal, than methylcholanthrene. Benzpyrene was definitely less carcinogenic in this vehicle and with this route of injection, as far as tumors of the lungs were concerned, than dibenzanthracene or methylcholanthrene.

The observations are of interest because benzpyrene is more carcinogenic than dibenzanthracene in producing subcutaneous sarcoma or carcinoma of the skin in mice, although less so than methylcholanthrene.[12] As a matter of fact, at the termination of the experiment, 9 of 11 mice receiving 1.0 mg. of methylcholanthrene and 4 of 10 mice receiving 0.5 mg. of methylcholanthrene had subcutaneous tumors at the sites of injection, whereas no subcutaneous sarcoma was encountered in the other groups.

It is apparent that with subcutaneously injected lard solutions of the three hydrocarbons there is no parallelism between the local carcinogenic power and the ability to produce tumors in the lungs. The reasons for the phenomenon are obscure, but are likely in part dependent on the physical state of the hydrocarbon, i. e., on the ability of the agent or its derivatives to be transferred from the locus of injection to the pulmonary tissue.

With all three compounds there is an accurate correlation between the dose of the hydrocarbon and the incidence and average number of induced pulmonary tumors. Thus, with 1.0 mg. of dibenzanthracene all animals had an average of 4.4 tumors; with 0.5 mg., the mice had an average of 2.8 tumors and with 0.25 mg., 70 per cent of the mice had an average of 1.8 tumors. The carcinogenic index in this instance, with the time being constant, is derived by multiplying the percentage of animals in which tumors develop by the average number of tumors per tumor-bearing animal; the agreement between the dose and the index values is evident.

Experiment 7. Time of Appearance of Pulmonary Tumors with Methylcholanthrene in Fatty Solvents.—In the course of investigations on the effect of various solvents on carcinogenesis with methylcholanthrene,[13] 53 strain A male mice each received a subcutaneous injection of 0.5 mg. of methylcholanthrene dissolved in 0.25 cc. of lard, tricaprylin, tricaproin, tricaprylin-trilaurin mixture or mouse fat. Each animal was killed as soon as an indubitable subcutaneous tumor appeared, and the lungs were examined for the presence and the number of pulmonary tumors.

The results, as far as tumors of the lungs were concerned, were identical with the different solvents and are summarized as one group in table 7. It is seen that tumors of the lungs began to appear in ten to twelve weeks in mice given 0.5 mg. of methylcholanthrene in fatty solvents, and that the number of tumor-bearing animals as well as the average number of pulmonary tumors per mouse rose as the time after administration increased.

TABLE 7.—*Experiment 7. Time of Appearance of Pulmonary Tumors in Strain A Mice Given Subcutaneously 0.5 Mg. of Methylcholanthrene in Various Solvents*

Time, Weeks	Mice Killed	Number with Tumors of the Lungs	Percentage with Tumors of the Lungs	Average Number Tumors per Mouse
8	6	0		..
10	10	0	0	..
12	12	5	40	2
14	5	4		3
16	4	2	66	4
18	3	3		4
20	4	3	85	3
22-30	9	9	100	6

These data are comparable with the findings with subcutaneous injection of 0.5 mg. of methylcholanthrene dispersed in horse serum (experiment 5). Pulmonary tumors appeared earlier and in greater number with the latter preparation, probably because of the greater diffusibility and ability to come in contact with the lung tissues.

A group of 10 strain A mice 2 months old, which are not included in the summary, were given subcutaneously 0.1 mg. of methylcholanthrene in 0.25 cc. of lard. They were killed six months later; 5 animals had single pulmonary tumors, and 2 had 2 pulmonary tumors each. The incidence of 70 per cent at eight months was sufficiently high to prove that this dose induced pulmonary tumors.

COMMENT

The induction of primary pulmonary tumors in animals is dependent on many factors, which can be divided into (1) the susceptibility of the animal and (2) the carcinogen.

13. Shimkin, M. B., and Andervont, H. B.: Pub. Health Rep., to be published.

1. The susceptibility of the animal, a characteristic which is probably genetically determined, is of two types: general susceptibility to the carcinogen and susceptibility of a specific organ. Thus, rabbits are extremely resistant to carcinogenic aromatic hydrocarbons,[14] while mice are extremely susceptible. Susceptibility of a specific organ can be illustrated by the failure to produce tumors of the lungs in rats by the intratracheal insufflation of methylcholanthrene,[15] while a similar procedure with strain A mice induced tumors of the lungs within five months;[11] both species are susceptible to subcutaneous sarcogenesis with the agent.

The inbred strains of mice show a marked difference in their susceptibility to induced pulmonary tumors,[16] and this indicates the possible mode of action of the carcinogens on lung tissue. It has been demonstrated by means of subcutaneously injected dibenzanthracene[16a] and intravenously injected methylcholanthrene[8] that the strains of mice which are most susceptible to the development of spontaneous pulmonary tumors are also most susceptible to the induction of these tumors with the hydrocarbons, and vice versa. This suggests that the compounds are accelerators of some process inherent and genetically determined in the animals. It is true that tumors of the lungs can be induced in all strains of mice, including the resistant ones,[4h] but no strain of mouse which has been adequately studied at advanced ages is completely free of spontaneous occurrence of this type of neoplasm.[17]

In strain A mice the susceptibility to spontaneous development of tumors of the lungs has been shown to be a dominant genetic characteristic;[2b] this is also true of the induced tumors.[4b, e]

2. The factors concerning the carcinogen in the induction of pulmonary tumors can be summarized under the following headings:

(*a*) The substances which have been found to produce pulmonary tumors in mice include tar, probably owing to its benzpyrene content, the three common polynuclear aromatic hydrocarbons, methylcholanthrene, dibenzanthracene and benzpyrene, 8,9-dimethyl-1,2-benzanthracene,[18] and 3,4,8,9-dibenzpyrene.[18a] Two compounds which are not hydrocarbons, 3,4,5,6-dibenzcarbazole and 2-amino-5-azotoluene, also have been reported[41] to produce pulmonary tumors in strain A mice.

(*b*) The dose of the carcinogen influences the appearance of pulmonary tumors in mice. In this report it has been shown that the

14. (*a*) Burrows, H., and Boyland, E.: Am. J. Cancer **32**:367, 1938. (*b*) Klinke, J.: Ztschr. f. Krebsforsch. **47**:341, 1938.

15. Valade, P.: Compt. rend. Acad. d. sc. **204**:1281, 1937.

16. (*a*) Andervont, H. B.: Pub. Health Rep. **53**:1647, 1938. (*b*) Shimkin.[8]

17. Little, C. C.; Murray, W. S., and Cloudman, A. M.: Am. J. Cancer **36**: 431, 1939.

18. Shear, M. J.: Am. J. Cancer **28**:334, 1936.

18a. Kleinenberg, H. E.: Arch. Biol. Sc. (U.S.S.R.) **51**:127, 1938.

number of animals showing tumors, the average number of tumors per animal and the time of appearance are directly proportional to the amount of hydrocarbon employed (fig. 3 and table 6). Lettinga,[19] using a more resistant strain of mice than the A strain, found that five subcutaneous injections of 0.5 mg. of dibenzanthracene produced multiple pulmonary tumors in the animals, that 0.05 mg. injected five times raised the incidence of single pulmonary tumors and that doses below this were ineffective. The abrupt transition from a small to a large number of induced tumors suggests an overflow of the carcinogen from the actual site of injection as the cause of the genesis of tumors of the lungs.[20]

In strain A mice an intravenous injection of 0.05 mg. of methylcholanthrene or a subcutaneous administration of 0.1 mg. in lard induces pulmonary tumors. In this connection, dibenzanthracene injected subcutaneously in doses ample to induce tumors of the lungs has not been detected in the lungs of these animals.[21] The dose necessary to evoke pulmonary tumors in susceptible mice is therefore extremely small, or else the hydrocarbon undergoes alterations which destroy its absorption spectrum bands.

(*c*) The route by which the carcinogen is given modifies the induction of pulmonary tumors. Some of the methods of administration that have been used by various investigators are: repeated application of coal tar to the skin;[22] inhalation of dust containing tar;[23] subcutaneous injection of carcinogenic hydrocarbons,[16a] dibenzcarbazole or azotoluene;[41] intravenous injection of serum or charcoal dispersions of carcinogenic hydrocarbons;[4d] injection of the hydrocarbons into the peritoneal[24] or pleural cavity[25] or into the spleen;[26] insertion of a string impregnated with dibenzanthracene through the lung;[4d] intratracheal injection of the hydrocarbons dispersed in horse serum;[11] feeding large doses of dibenzanthracene emulsion in olive oil,[27] or injection of dibenzanthracene in olive oil into the stomach by means of a tube.[28] In the last method mentioned, there is direct aspiration of the carcinogen into the lungs.[28]

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19. Lettinga, T. W.: De carcinogene werking van kleine doses 1,2,5,6-dibenzanthraceen, Academisch., Proefschrift, Amsterdam, 1937.
20. Kennaway, E. L., and Kennaway, N. M.: Acta, Union internat. contra cancer **2**:101, 1937.
21. Lorenz, E.: Unpublished data.
22. Murphy, J. B., and Sturm, E.: J. Exper. Med. **42**:693, 1925.
23. Campbell, J. A.: Brit. J. Exper. Path. **15**:287, 1934.
24. Schabad, L. M.: Acta, Union internat. contra cancer **3**:369, 1938.
25. Andervont, H. B., and Lorenz, E.: Pub. Health Rep. **52**:1931, 1937.
26. Furth, J., and Furth, O. B.: Am. J. Cancer **34**:169, 1938.
27. Lorenz, E., and Stewart, H. L.: Unpublished data.
28. Magnus, H. A.: J. Path. & Bact. **49**:21, 1939.

The most efficacious technic of inducing pulmonary tumors in mice, as far as the rapidity of appearance and multiplicity are concerned, is that of intravenous injection. Fewer tumors, arising later, are obtained with the subcutaneous and the intratracheal mode of administration.

(*d*) The medium in which the carcinogens are introduced into the animals also modifies their action. The production of pulmonary tumors is accelerated when a carcinogen is injected in a readily soluble form, as in dog or horse serum dispersions.[4d] Various solvents for the hydrocarbons, such as lard, glycerin, mouse fat, tricaproin and tricaprylin, cause fewer and later-appearing pulmonary tumors than the serum preparations. Moreover, the medium exerts further effects, the explanation of which is obscure: The relative carcinogenicity of methylcholanthrene and dibenzanthracene are reversed when the compounds are injected as lard solutions rather than as dispersions in serum.

When the hydrocarbons are maintained firmly at the site of injection by the menstruum, as is true when they are in cholesterol pellets or adsorbed on charcoal,[29] few pulmonary tumors are produced. The role of the medium for the carcinogen is important evidence in support of the contention that pulmonary tumors in mice are induced through direct contact of the compound or of its active derivatives with the susceptible pulmonary tissue.

(*e*) The time of appearance of the induced pulmonary tumors depends on the susceptibility of the mouse, the carcinogen employed, the dose, the medium for the compound and the route of injection. With 1.5 mg. of methylcholanthrene injected intravenously, 100 per cent of strain A mice have multiple tumors of the lungs in four weeks. With 0.5 mg. of dibenzanthracene injected intravenously, pulmonary tumors begin to appear in four weeks also, but the percentage of tumor-bearing mice and the average number of tumors per mouse are reduced. Grady and Stewart [9] found a single tumor in a strain A mouse thirty-two days after subcutaneous injection of 0.8 mg. of methylcholanthrene in lard, but at eleven weeks only 50 per cent of the animals had pulmonary tumors. In this investigation 0.5 mg. of methylcholanthrene in lard failed to induce pulmonary tumors before the tenth week, and in twelve weeks the incidence was 40 per cent. Thus, the greatest number of tumors in groups of strain A mice or in any one mouse appear earliest with the largest doses of intravenously injected methylcholanthrene; the time of appearance and the incidence are slightly longer and less, respectively, for intravenously injected dibenzanthracene and for either hydrocarbon administered subcutaneously.

SUMMARY

Spontaneous pulmonary tumors, usually single, appear in strain A mice 3 months old, and the incidence rises sharply with advancing age.

29. Andervont.[4d] Andervont and Lorenz.[25]

Intravenously injected 20-methylcholanthrene induces multiple pulmonary tumors within four weeks; the number of mice showing tumors and the average number of tumors per pair of lungs is proportional to the dose of the hydrocarbon. Intravenously injected 1, 2, 5, 6-dibenzanthracene induces pulmonary tumors as quickly as 20-methylcholanthrene, but the incidence in the group and the average number of tumors per mouse are significantly lower.

The definite difference in the power of 20-methylcholanthrene and 1, 2, 5, 6-dibenzanthracene to produce tumors of the lungs is obliterated when the compounds are injected subcutaneously, although the incidence in the group and the number of pulmonary tumors per mouse induced is still proportional to the dose of the hydrocarbon administered. Pulmonary tumors appear later with lard solutions of the compounds than with the colloid suspensions injected subcutaneously.

Lungs of strain A mice are an admirable tissue for many phases of investigations on the action of cancer-provoking chemicals.

PERICARDIAL MILK SPOTS

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Localized white opaque fibrous thickenings of the visceral pericardium (milk spots, tendinous patches, soldiers' spots, maculae tendineae, *Sehnenflecke,* etc.), most commonly seen on the anterior surface of the right ventricle, have been described and their origin disputed since at least 1806.[1] Their genesis has been held to be either inflammatory, mechanical or both, and there have been about equal numbers of adherents of the three concepts. There have been a few more or less successful experimental productions of these spots in animals. Numerous descriptions of the gross and microscopic appearance of the spots in man have been published, but data concerning their frequency, age distribution, anatomic location and association with various diseases are practically nonexistent, and it is therefore the purpose of this paper to present such data.

MATERIAL

The observations in this paper are derived from a series of 494 autopsies performed by myself [2] after I had become interested in this subject. Excluded from this number are 50 autopsies on infants under 1 year of age and 13 on older persons with obscuring lesions, such as diffuse pericarditis or pericardial adhesions. Only definite localized epicardial thickenings of 1 cm. or greater diameter (except for 6 between 7 mm. and 1 cm.) were included; the irregular thickenings frequently seen over the coronary vessels were not included.

GROSS OBSERVATIONS

Of the 494 persons 1 year or more of age, 170 (34.4 per cent) showed one or more pericardial milk spots (table 1). Of the 439 persons 18 years or more of age, 165 (37.6 per cent) showed spots, leaving the remaining 5 cases (9.1 per cent) in the group of 55 persons between 1 and 18 years of age (table 2).

Incidence by Age and Sex.—The distribution of milk spots by age groups is given in table 3. The incidence varies from 9.1 per cent in the

From the National Institute of Health, United States Public Health Service.

1. Tsunoda, T.: Frankfurt. Ztschr. f. Path. **3**:220, 1909.

2. The autopsies were performed at the University of Minnesota, and the microscopic sections were made there. The compilation of the data was done in Washington.

group from 1 to 18 years of age to 73.4 per cent in the group 80 or more years of age; the latter group is rather small, however, and throughout most of the life span, from 30 to 80 years, the incidence varies between 32 and 42 per cent. The groups in this age range are large enough to make the figures of value. If the production of milk spots were on a purely mechanical or an age basis, there should be a definite and marked increase of incidence with age; this series does not show such an increase.

In the literature the only figures for the incidence of milk spots are those of Tsunoda,[1] who stated that the incidence in the first decade was 8.5 per cent, in the second 10.0 per cent, in the third 23.0 per cent, in

TABLE 1.—*Incidence of Pericardial Milk Spots in Age Groups*

	18 Years and Over		1 to 18 Years		All Ages	
	Subjects	Percentage of Age Group	Subjects	Percentage of Age Group	Subjects	Percentage of Age Group
With spots	165	37.6	5	9.1	170	34.4
Without spots	274	62.4	50	90.9	324	65.6
Total	439	100.0	55	100.0	494	100.0

TABLE 2.—*Data on Subjects from One to Eighteen Years of Age*

Sex	Age, Years	Cause of Death	Size of Milk Spot	Location	Weight of Heart, Gm.
M	2½	Hydronephrosis	3 patches, largest 1 cm.	Various	70
M	5	Tumor of brain	3 mm.	Left ventricle	75
F	7	Burns	3 mm.	Left apex anterior	110
M	10	Lymphatic leukemia	7 mm.	Left ventricle anterior	140
M	15	Tumor of brain	1 cm.	Right ventricle anterior	180

the fourth 28.0 per cent, in the fifth 47.0 per cent, in the sixth 54.0 per cent and later than the sixth decade 65.0 per cent. Unfortunately, the number of cases on which this series was based was not given. Adami [3] stated simply that they are to be found in more than 14 per cent of all persons coming to autopsy, while Kaufmann [4] gives the percentage as 80.

It will be noted that the sex incidence of milk spots is fairly similar; males made up 62.7 per cent of the group with spots and 69.4 per cent of the group without spots. If the subjects 18 years of age and over are taken, the figures are even closer, being 64.7 and 69.1, respectively.

3. Adami, J. G., and Nicholls, A. G.: The Principles of Pathology, Philadelphia, Lea & Febiger, 1909, vol. 2, p. 141.

4. Kaufmann, E.: Pathology for Students and Practitioners, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, p. 13.

Number of Spots per Subject.—The number of spots found in each subject is given in table 4. It will be noted that multiple spots were somewhat more frequent than single spots. The terms in quotation marks in the table are taken from the notes made on the subjects.

Size of Spots.—As stated previously, the milk spots included in this series were 1 cm. or more in diameter in all except 6 cases. The exact number of spots of each size found cannot be given for the reason that in most of the numerous cases of multiple spots, only the size of the largest was noted. However, it is my estimate that in a large series of

TABLE 3.—*Incidence of Milk Spots by Age and Sex*

Age Group, Yr.	Number With Spots			Number Without Spots			Number in Age Group	Percentage of Age Group With Spots
	Males	Females	Total	Males	Females	Total		
Below 18	4	1	5	26	24	50	55	9.1
18-29	8	3	11	16	13	29	40	27.5
30-39	8	8	16	18	10	28	44	36.4
40-49	11	13	24	23	21	44	68	35.3
50-59	22	10	32	45	23	68	100	32.0
60-69	34	7	41	38	21	59	100	41.0
70-79	25	5	30	33	9	42	72	41.7
80 and over	6	5	11	4	0	4	15	73.4
All ages	118	52	170	206	121	324	494	34.4
18 and over	114	51	165	177	97	274	439	37.6

TABLE 4.—*Number of Spots per Subject*

Number of Spots	Subjects
Unspecified	12
One	62
Two	15
Three or "few"	28
"Several"	30
"Numerous" or "extensive"	23
Total	170

milk spots, the sizes 1 cm., 1.5 cm., 2 cm., 2.5 cm., 3 cm. and over 3 cm. in diameter will comprise about 30, 30, 20, 10, 5 and 5 per cent, respectively.

Location of Spots.—Table 5 shows that the most common location is on the anterior surface of the right ventricle. Where the spots were numerous and extensive, the largest and thickest would more often be in this area also. The table is not complete because in about 30 cases spots were merely stated to be present, and in the cases with numerous spots each location was not noted. On the other hand, frequently cases with two or three spots will have them in one location.

Summarizing, it will be seen from table 5 that spots were noted on the right side of the heart 101 times, against 35 on the left, on the anterior surface 76 times, against 27 on the posterior, and on the ventricles 116 times, as compared with 17 on the atria.

Association of Spots with Disease.—Pericardial milk spots have not been definitely associated with any certain disease or groups of diseases. Tsunoda [1] stated that they are commonest in hypertrophied or dilated hearts, and Girgensohn [5] stated that in rheumatic conditions and with

TABLE 5.—*Location of Spots*

Location	Cases
"Numerous" or "extensive"	23
Right ventricle	
Anterior (including pulmonary conus)	54
Posterior	22
Unspecified	9
Left ventricle	
Anterior	22
Posterior	5
Unspecified	7
Right atrium	16
Left atrium	1
Intrapericardial aorta	8
Venae cavae	5

TABLE 6.—*Incidence in Various Disease Groups*

Group	With Spots		Without Spots		Total	
	No.	%	No.	%	No.	%
All subjects 18 or more years of age	165	37.6	274	62.4	439	100.0
First group less 42 with valvular heart disease	137	34.5	260	65.5	397	100.0
First group less 26 with severe coronary sclerosis	152	36.8	261	63.2	413	100.0
First group less 121 with enlargement of the heart	107	33.7	211	66.3	318	100.0
First group less 33 with hypertension	151	37.2	255	62.8	406	100.0
Chronic valvular heart disease	28	66.7	14	33.3	42	100.0
Severe coronary sclerosis	13	50.0	13	50.0	26	100.0
Enlargement of the heart	58	47.9	63	52.1	121	100.0
Hypertensive heart disease	14	42.4	19	57.6	33	100.0

healed or recurrent inflammation in the heart they are increased; neither author gives actual figures. From table 6 it seems definite that there is an increase in the number of spots with chronic valvular heart disease (old and recurrent rheumatic, subacute bacterial and syphilitic), 66.7 per cent of the subjects with valvular heart disease showing them, as compared with 34.5 per cent for subjects without valvular disease. Increases with severe coronary disease and cardiac hypertrophy seem fairly definite, while an increase with hypertension is uncertain. The figures

5. Girgensohn, H.: Virchows Arch. f. path. Anat. **293**:73, 1934.

are based on the 439 subjects 18 or more years of age; the differences would be slightly greater if the entire group were used. Hearts were considered enlarged when they weighed over 400 Gm. in men and 350 Gm. in women.

The distribution of the 42 cases of valvular disease (old and recurrent rheumatic, subacute bacterial and syphilitic) was as shown in table 7. It will be seen that the different types of lesions show about the same incidence of spots.

Relation to Pleural Adhesions.—The presence and the degree of pleural adhesions were noted in 100 subjects with and 100 without spots, to see if there was any increase in the incidence of adhesions in the group with spots. Only old fibrous adhesions were considered. Of the 100 subjects with pericardial milk spots, 54 also had pleural adhesions, in 25 of whom the adhesions were extensive enough to involve one half or more of the total pleural surface. Of the 100 subjects without milk spots, 38 had pleural adhesions, and in 20 of these the adhesions were

TABLE 7.—*Distribution of Cases of Valvular Disease with Regard to Spots*

	Mitral Valve		Aortic Valve				Other Valves
	Old or Recurrent Rheumatic Lesion	Subacute Bacterial Lesion	Old or Recurrent Rheumatic Lesion	Subacute Bacterial Lesion	Calcified Nodular Lesion	Syphilitic Lesion	Subacute Bacterial Lesion
With spots.....	11	3	5	1	5	2	1 (pulmonary)
Without spots.....	7	1	3	0	1	1	1 (tricuspid)

extensive. Thus, there appears to be a slight increase in pleural adhesions in the group with pericardial milk spots.

Size of Heart.—The mean weight of the hearts from the 165 subjects 18 or more years of age in whom pericardial milk spots were present was 378 Gm.; that of the hearts from the 274 subjects in whom pericardial milk spots were not present was 352 Gm.

Parietal Pericardium.—Thickenings of the parietal pericardium, similar in appearance and distribution to those on the visceral pericardium, occurred frequently, although less so than the latter. No special study of the parietal thickenings was made.

EXPERIMENTAL PRODUCTION

This was first attempted by Tsunoda,[1] who introduced pyroxylin, glass or rubber foreign bodies under the sternums of dogs and rabbits (number not stated), and then examined the pericardium after intervals of one week to thirteen months. During the first two postoperative months the pericardium was essentially normal, but after four to six

months it showed cloudy areas grossly and a slight increase in collagen microscopically; white collagenous areas appeared in from six to thirteen months. Ishisaki [6] produced epicardial thickenings in 24 of 26 guinea pigs by constricting the thorax with a plaster cast. Most of these epicardial thickenings were slight in degree, but a few were marked. There were also thickenings of the parietal pericardium. Good gross and microscopic descriptions and illustrations are given. Girgensohn [5] injected horse serum intrapericardially into sensitized rabbits through an operative incision, then killed them six to thirteen weeks later. Pericardial thickenings were found in 5 of the 10 animals. Whether similar lesions might have been produced by operative intrapericardial injection, with its unavoidable trauma, in unsensitized animals was not mentioned.

MICROSCOPIC OBSERVATIONS

Detailed microscopic descriptions of pericardial milk spots are to be found in the literature,[7] and there is no intention to duplicate them in this paper. I will give only a few observations from the study of 132 spots in this series which were microscopically sectioned. Through their thickest portions, 27 spots were from 100 to 200 microns thick, 76 were from 200 to 350 microns, 19 were from 350 to 500 microns, and 10 were over 500 microns thick; these are approximate measurements, made by comparing the thickness of the milk spot with a microscopic field of a known width of 440 microns. Small to large collections of lymphoid cells just underneath the fibrous spots were frequent, and fairly large numbers of lymphoid cells were present within the spots in several instances. Eleven spots showed serosal epithelium enclosed within or penetrating into the collagenous tissue in glandular or canalicular formation. Slight to moderate palisading of fibroblastic cells just under the serosal epithelium was seen in 9 cases. Long frondlike villi projecting into the pericardial cavity were present in 4 cases, and shorter villi or overhanging ends of collagenous masses were present in 11 more. Frequently more than one of these atypical features were present. These appearances, present in 15 or 20 per cent of this series of milk spots, suggest or are transitions from an inflammatory process to the usual type of milk spot. They were not seen in any particular type of case, but with all types.

SUMMARY

Pericardial milk spots occurred in 170 (34.4 per cent) of 494 persons 1 year or more of age. In 439 persons 18 or more years of age the incidence was 37.6 per cent. In general, there is an increase of inci-

6. Ishisaki, S.: Virchows Arch. f. path. Anat. **244**:214, 1923.

7. Tsunoda.[1] Ribbert: Virchows Arch. f. path. Anat. **147**:193, 1897.

dence with age, but this increase is by no means rectilinear. The spots are scarce in children and very frequent in old age, but between 35 and 75 years of age there is little change in incidence.

There seems to be a definite association with chronic or recurrent valvular heart disease; of 42 persons with such disease, 28 (66.7 per cent) showed spots. Patients with severe coronary sclerosis and enlarged hearts showed fairly definite increases (50.0 and 47.9 per cent, respectively).

The occurrence of more than one spot is slightly more frequent than that of only one. Spots occur on the right side, anteriorly, and on the ventricles much more frequently than they do on the opposite surfaces.

Old pleural adhesions are slightly more frequent in patients with spots than in those without.

Fifteen or 20 per cent of spots show appearances (projecting villi, cellular exudation, subepithelial palisading or epithelial enclosures in collagenous tissue) which suggest transitions from a more active inflammatory process to the usual type of milk spot.

Case Reports

MALIGNANT GRANULOSA CELL TUMOR OF THE OVARY

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Although a large number of granulosa cell tumors of the ovary have been reported, especially in recent years, as granulosa cell carcinoma, it is doubtful whether the name "carcinoma" should be universally applied to them. A majority of the reports deal with tumors removed at operation. Only a few of the patients have been followed for a period of five years after operation, and there are no detailed postmortem reports on those whose death was due to metastases.

The diagnosis of malignancy has been based almost entirely on the microscopic appearances in surgical specimens. Novak [1] estimates the degree of malignancy as slight, with some 5 to 10 per cent recurring and metastasizing, a few being highly malignant. Meyer [2] in following up 33 patients found that 3 had died from metastases, but he gave no details. Habbe [3] in following up Meyer's 33 patients found that there had been 5 deaths (2 postoperative) and 4 recurrences. Klaften [4] reported that in a group of 80 collected instances recurrences or metastases were present in 4, i. e., 5 per cent. Novak and Brawner [5] stated that of 32 patients, 6 showed unmistakable signs of a malignant growth at the time of operation and 3 had recurrences and that therefore 28.1 per cent of the growths were malignant. Schiller [6] in a study of 24 granulosa cell tumors concluded that from the microscopic standpoint 8 were malignant. Novak and Gray,[7] studying 42 tumors, found that the degree of malignancy as estimated by microscopic observation was less than in ovarian carcinomas as a whole. Taussig [8] stated that such tumors were usually unilateral and showed no tendency to metastasize.

Von Werdt [9] reported an instance in which in a patient who died three and one-half months after operation no metastases were found at necropsy. Voigt's [10] patient died about six months after operation with evidences of recurrence, but no autopsy was made. One of Neu-

From the Department of Pathology, University of Minnesota.

1. Novak, E.: Am. J. Obst. & Gynec. **26**:505, 1933. Novak, E., and Long, J. H.: J. A. M. A. **101**:1057, 1933.
2. Meyer, R.: Arch. f. Gynäk. **145**:2, 1931.
3. Habbe, E.: Zentralbl. f. Gynäk. **55**:1088, 1931.
4. Klaften, E.: Arch. f. Gynäk. **150**:643, 1932.
5. Novak, E., and Brawner, J. N.: Am. J. Obst. & Gynec. **28**:637, 1934.
6. Schiller, W.: Pathologie und Klinik der Granulosazelltumoren, Vienna, Wilhelm Maudrich, 1934.
7. Novak, E., and Gray, L. A.: Am. J. Obst. & Gynec. **31**:213, 1936.
8. Taussig, F. J.: Am. J. Cancer **15**:1547, 1931.
9. von Werdt, F.: Beitr. z. path. Anat. u. z. allg. Path. **59**:453, 1914.
10. Voigt, M.: Arch. f. Gynäk. **70**:87, 1903.

mann's [11] patients died six months after operation with recurrence of the tumor. Aschner's [12] patient also died about six months postoperatively with signs of recurrence, but there was no postmortem examination.

Arnold, Koerner and Mathias [13] reported the following case: A woman 68 years of age, with a granulosa cell tumor, was first operated on for an ovarian tumor in 1908. She had an incomplete operation for the removal of a recurrence in 1924 and finally died in 1928 of "carcinosis universalis."

Te Linde [14] searched the literature for the end results in granulosa cell tumor and found that of 17 patients followed, 13 were reported as well for from one to eleven years after operation. Of these 13, 2 only were followed for as long as five years—one, reported by Isbruch,[15] was a woman aged 50 years, who was still alive and well eleven years later, and the other, reported by Müllerheim,[16] was a woman aged 69 years, who was still well eight years after the operation.

Schiller included 16 granulosa cell tumors in his benign group. Of the patients, 4 were alive and well five years, 1 nine years and 1 ten years after operation. Another died one month after operation, of pulmonary embolism, and at postmortem examination had metastases in the left kidney.

A third case of survival for more than five years may be added. This concerned a woman aged 20 years who was operated on on May 6, 1932, and an ovary partially replaced by a tumor 2 cm. in diameter removed. This tumor was in part typically follicular and in part cylindroid. The patient was alive and well, without evidence of recurrence, on Nov. 15, 1938.

Fauvet [17] reported 8 instances of granulosa cell tumor. One patient died postoperatively of pulmonary embolism, and it was found that the ovarian tumor had perforated its capsule and spread regionally; in another patient recurrence took place. Soltmann's [18] patient died ten days after operation from paralytic ileus; metastases were present in the sacrum. Schulze [19] reported 4 instances of granulosa cell tumor and mentioned that in 1 instance death with recurrence took place four years after operation; autopsy was apparently not done.

Of the 8 patients with malignant tumors reported by Schiller and mentioned in a foregoing paragraph, 2 showed metastases at the time of operation, a third died four days after operation from pulmonary embolism and showed at postmortem examination peritoneal metastases, while a fourth died four months after operation with generalized metastases. On the last an autopsy was apparently not done.

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11. Neumann, H. O.: Virchows Arch. f. path. Anat. **258**:284, 1925.
12. Aschner, B.: Arch. f. Gynäk. **115**:350, 1922.
13. Arnold, W.; Koerner, J., and Mathias, E.: Virchows Arch. f. path. Anat. **277**:48, 1930.
14. Te Linde, R. W.: Am. J. Obst. & Gynec. **20**:552, 1930.
15. Isbruch, F.: Zentralbl. f. Gynäk. **50**:89, 1926.
16. Müllerheim, R.: Zentralbl. f. Gynäk. **52**:689, 1928.
17. Fauvet, E.: Zentralbl. f. Gynäk. **56**:3088, 1932.
18. Soltmann, C. H.: Virchows Arch. f. path. Anat. **284**:466, 1932.
19. Schulze, M.: Am. J. Obst. & Gynec. **26**:627, 1933.

Since there have been so few instances of this disease reported in which the final outcome was ascertained by autopsy, it seems worth while to place such a case on record.

REPORT OF A CASE

Uterine curettings from a woman aged 59 years were submitted for examination, Feb. 19, 1932, and a diagnosis was made of hypertrophic endometrium (fig. 1 *A*). No history was obtained at that time.

August 19 specimens of uterus and ovarian tumor were sent for examination. On this occasion the following history was obtained: The patient's mother died of cancer of the breast. The patient had borne 2 children. The menopause took place in 1918 (fourteen years before). There was no subsequent bleeding until about one year before the last admittance to the hospital. The bleeding was entirely without regularity; at times there was spotting every four or five days, and there were periods as long as eight weeks with no hemorrhage. At times many large clots were passed. The most severe of all the hemorrhages had taken place August 16. On admission on that day the patient complained of a sharp pain radiating down the left leg. She had lost about 16 pounds (7.3 Kg.) in weight. There was the additional history of the removal of a cervical polyp about ten months before; curettage had been done the preceding February, followed with treatment by radium. Examination showed an obese woman with distinct evidences of anemia. The hemoglobin content was 45 per cent; the red blood cells, 3,230,000. The blood pressure ranged between 180 systolic and 90 diastolic and 154 systolic and 82 diastolic. The cervix was lacerated and cystic.

At operation on August 19 the left ovary contained a hemorrhagic cyst, about 20 cm. in diameter; the uterus was about twice the normal size, with a fibrous myometrium and many myomas. The endometrium was irregularly thickened. Subtotal hysterectomy and left salpingo-oophorectomy were done.

Sections of the ovary showed numerous cysts, most of them with no epithelial lining. There were large areas of necrosis and old and fresh hemorrhages. Some remnants of ovarian tissue were present along one edge. The relatively solid portion of the tumor was made up of solid cellular areas, the cells of which in places assumed a cordlike arrangement. In places small rounded spaces were present, which resembled follicles. The cells stained fairly uniformly but were of a variety of shapes: polyhedral, rounded, elongated and flattened. Scattered through the tumor were fibrous trabeculae, usually hyaline, which did not divide the tumor cells into nests. Mitotic figures were not found (fig. 1 *B*). Sections of the uterus showed adenomyoma. There was cystic, hypertrophic endometrium. A diagnosis was made of carcinoma of the ovary, with the suggestion that it was of the granulosa cell type.

The patient was not seen again until May 27, 1937, at which time she came under the care of another physician. She complained of a sensation of fulness in the epigastrium, constipation and edema of the ankles. She stated that following the operation she was in good health until about one year before, when she noted swelling of the ankles. In December 1936 she had "influenza" with vomiting, headache and general malaise. Since that time she had had the sensation of fulness in the upper part of the abdomen; it bore no relation to meals. There had been no loss of weight. Physical examination revealed pitting edema of the ankles and a large, freely movable, apparently cystic mass, about the size of a full term pregnancy, filling the abdomen. At operation a very large multilocular cystic tumor, apparently arising from the right ovary and adherent to the adjacent

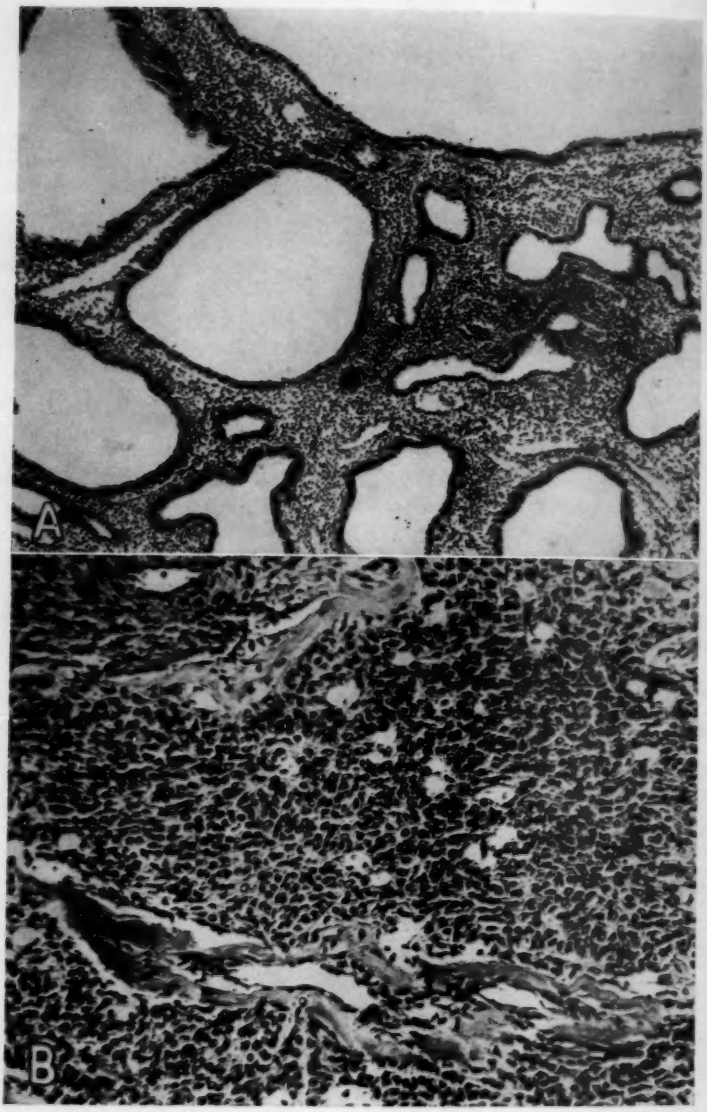

Fig. 1.—*A*, cystic, hyperplastic endometrium, removed Feb. 19, 1932. *B*, low power photomicrograph of the right ovarian tumor, removed Aug. 19, 1932.

structures, was found. The adhesions were not dense. A number of other small cysts were also present, but the general peritoneal surfaces seemed not to be involved. The entire mass was removed. A portion of it was sent for examination. This showed numerous large cysts and some solid areas.

The sections showed a tumor somewhat resembling that found in the left ovary but not so well differentiated. There were roughly rounded oval cellular areas, separated from one another by narrow and broad masses of loose tissue, somewhat resembling areolar connective tissue. The cellular masses were not sharply bounded and seemed to fade into the other type of tissue. Higher magnification showed that the cellular areas were composed of fairly uniform cells, indistinctly arranged in cords. Occasional structures suggesting follicles were observed. No mitotic figures were found. There were no glands or recognizable ovarian tissue (fig. 2 *A*). A diagnosis was made of carcinoma of the ovary.

The patient was next seen July 10, 1938, when the history was obtained that after the second operation she felt well until the preceding winter, when weakness of the left leg and spells of dizziness were noted. She complained of pain in the right side of the head and difficulty in hearing in the right ear. During recent weeks there had been spells of a projectile type of vomiting and abdominal pain. Polyphagia was also complained of. She seemed to understand what was said to her but either would not or could not answer. The blood pressure at this time was 190 systolic and 100 diastolic. Some fever was present, and in succeeding days the temperature varied from 104 to 105.5 F. Death occurred July 15. The age at this time was 65 years.

Postmortem examination, done twelve hours after death, showed a well nourished white woman. Two old scars were present on the abdominal wall but no evidences of tumor in either. There was edema of the ankles and over the sacrum. The abdominal fat was 5 cm. in thickness. Scattered fibrous adhesions were present in the peritoneal cavity. The appendix was small, fibrous and nonadherent. Throughout the peritoneal cavity were brownish red to gray nodules, of fleshy consistence and varying from 0.5 to 2 cm. in diameter. They were present on the parietal and visceral layers of the peritoneum and in the mesentery. Attached to the posterior parietal peritoneum between the liver and the spine was a firm, grayish brown pedunculated mass, 2 by 3 cm.

The heart weighed 400 Gm. and was normal except for some myocardial fibrosis. The lungs showed a small amount of purulent bronchitis and bronchopneumonia. The spleen was of normal size. The liver disclosed evidences of chronic passive congestion. The gallbladder, gastrointestinal tract, pancreas and adrenals were normal. The kidneys had finely granular surfaces.

The pelvic structures showed no evidences of tumor. The stump of the cervix was 2.5 cm. in diameter and contained a single cyst, filled with gelatinous material.

An encapsulated nodule, 1 cm. in diameter, was present in the right breast. A calcified mass, 3 cm. in diameter, was present in the left breast.

The scalp, calvarium and meninges appeared normal. Cross sections of the brain showed that the anterior portion of the right lateral ventricle was almost completely filled by a reddish gray, soft, well outlined, partially necrotic mass, 3.5 by 2.75 by 2.75 cm. It involved the lower portion of the corpus callosum and protruded into the left lateral ventricle. The right caudate nucleus and the superior aspect of the right internal capsule were also involved. In the anterior portion of the left parietal lobe was a firm gray nodule about 7 mm. in diameter. The microscopic observations were as follows: The mesenteric lymph node showed the normal

tissue entirely replaced by tumor tissue, which for the most part was composed of irregular islands having a distinct cordlike arrangement (fig. 2 *B*). The diffusely cellular parts had appearances much like those of the tissue removed at the second

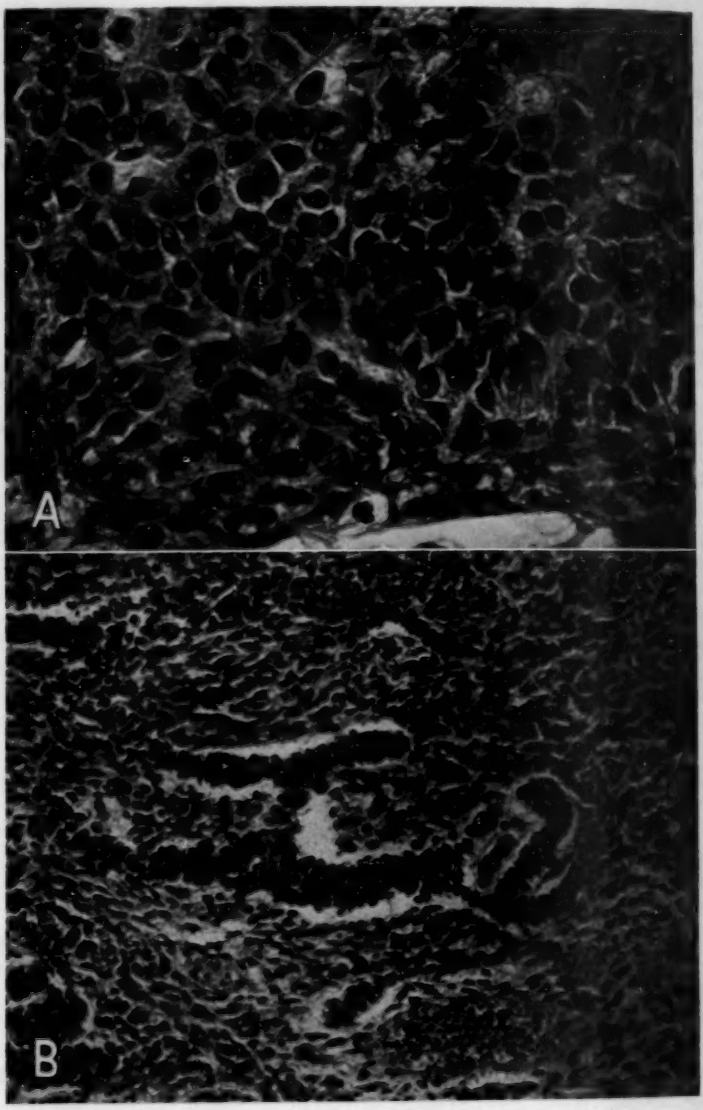

Fig. 2.—*A*, high power photomicrograph of the tumor of the left ovary, removed in May 1937. *B*, high power photomicrograph of a metastasis in a lymph node. The normal structure of the node is entirely replaced by tumor, partly in the form of diffuse cellular sarcoma-like tissue, partly in the form of twisted cords.

laparotomy, but with no cysts. Structures suggesting follicles were occasionally found. No mitotic figures were present.

The peritoneum structurally took the form of twisted cords of cells separated by spaces somewhat suggestive of gland spaces (fig. 3 *A*).

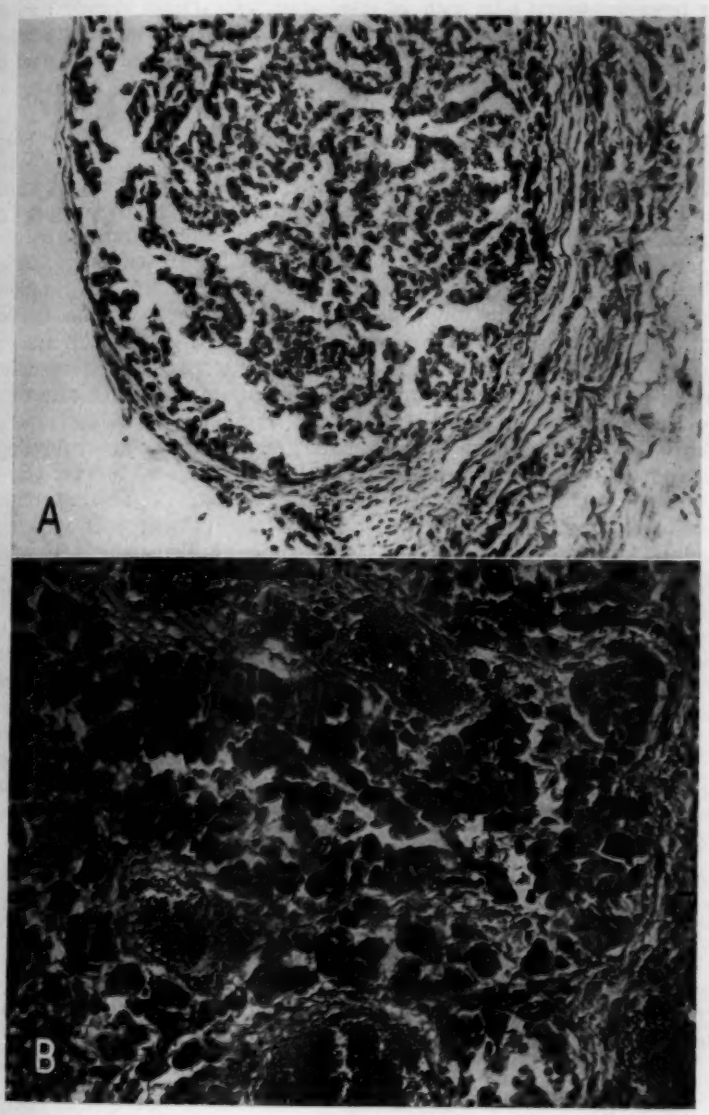

Fig. 3.—*A*, photomicrograph of a peritoneal metastasis. Note the cylindroid arrangement of the cells in twisted cords. *B*, photomicrograph of a metastasis in the brain. Note the large cells in the form of solid cords with only a slight tendency toward follicle formation.

Sections from the two tumors in the brain showed appearances which did not very closely resemble one another or those of the tumors elsewhere in the body. They showed a very great polymorphism of the cells with rather numerous multinucleated giant cells. One section (fig. 3*B*) disclosed large cordlike masses of rather large polyhedral cells with, in some areas, appearances vaguely reminding one of follicles. The other tumor was made up of a tissue having a rather fibrous structure with comparatively small and scattered cells. A number of giant cells were present, some of which appeared like rosettes. Follicle-like structures were hard to find, unless the rosettes are to be considered follicles.

The tumors of the breasts were found to be fibroadenoma.

The origin of granulosa cell tumors has been discussed by many authors, and the majority accept Meyer's opinion that they come from cell rests and not from adult granulosa cells. Meyer recognizes three types, folliculoid, cylindroid and diffuse, the last resembling sarcoma, all of which may coexist in the same tumor. There are also numerous papers dealing with the physiologic effects of the secretion of these tumors. During sexual life there may be amenorrhea or irregular menstruation. The usual effect after the menopause is a recurrence of uterine bleeding. Such tumors developing in young children cause precocious puberty.

The case reported is an instance of a granulosa cell tumor developing about fourteen years after the menopause. It produced uterine bleeding and a typical hyperplastic, cystic endometrium. Following removal of the ovary which contained the tumor the patient was well for a period of almost five years. At this time another tumor developed in the opposite ovary. This second tumor also had the structure of a granulosa cell tumor but was not quite identical with the first. It is probable that the second tumor represents a metastasis from the first. Although the granulosa cell tumor is usually unilateral, several authors have reported examples of a bilateral tumor (Rummeld;[20] Klaften[4]). Following removal of the second ovarian tumor the patient had a period of good health, which lasted for only a few months, after which symptoms of an intracranial lesion developed. Post mortem metastases were found in the peritoneum, the mesenteric lymph nodes and the brain.

SUMMARY

A granulosa cell carcinoma of the ovary is reported, which developed fourteen years after the menopouse and produced cystic hyperplasia of the endometrium with uterine bleeding. Five years after removal of the tumor there appeared signs of involvement of the opposite ovary. This was also removed. Death occurred one year later or six years after the first operation. Postmortem metastases were found in the mesenteric lymph nodes, the peritoneum and the brain.

An instance of a patient alive and well six and one-half years after removal of a granulosa cell tumor is also reported.

The diagnosis of granulosa cell carcinoma should be reserved for the tumors of granulosa cell type which are definitely malignant.

20. Rummeld, P.: Zentralbl. f. Gynäk. **55**:292, 1931.

SYMMETRIC NECROSIS OF THE GLOBUS PALLIDUS IN BARBITURATE POISONING

A. De Groat, M.D., Detroit

Necrosis in the basal ganglions from intoxication is commonly produced by no agent other than carbon monoxide. This is remarkable in view of the fact that carbon monoxide probably acts only through anoxemia. Asphyxia from other causes, including depressant drugs, shows no such selective action on the basal ganglions; rather it produces diffuse lesions both in man and in experimental animals.

However, Gonzales, Vance and Helpern [1] reported a case of symmetric softening of the globus pallidus due to barbital poisoning which seems to be unique in the literature. I have recently encountered a similar case which is reported here for its theoretic and medicolegal interest.

REPORT OF A CASE

The subject was a white woman 24 years old, a nurse, apparently in good health. She had attended a social function and had retired at a normal hour. The next morning she was discovered by her roommate to be unconscious. There were marked cyanosis and feeble respiration. The cyanosis deepened, and the breathing became irregular, necessitating artificial respiration at intervals over a period of about six hours. The reflexes were absent but returned slowly over a period of about two days. The patient remained unconscious until her death five days later, from bronchopneumonia.

From a further investigation of her history it was apparent that she was addicted to a stupefacient of some kind, and pentobarbital sodium was found among her personal effects.

At necropsy the chief anatomic changes were edema of the brain, symmetric softening of the globus pallidus, bronchopneumonia and fatty degeneration of the liver. There was a small amount of calcification in the arterioles of the corpus striatum, and degeneration of ganglion cells of varying degrees was found throughout the brain.

On the basis of what is known of the toxic properties and effects of carbon monoxide one may conclude that necrosis of the globus pallidus occurs when there is prolonged deep asphyxia followed by temporary survival for several days. It thus appears that the same lesion may result from the action of barbiturates when these chance to produce the same set of circumstances. It is even conceivable that the lesion might follow strangulation, nitrogen monoxide anesthesia, the syncope of Stokes-Adams disease and irritation of the carotid sinus. An alternative theory is that carbon monoxide and the barbiturates exert a specific effect on the globus pallidus, but for this there is little evidence.

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1. Gonzales, T. A.; Vance, M., and Helpern, M.: Legal Medicine and Toxicology, New York, D. Appleton-Century Company, Inc., 1937.

Laboratory Methods and Technical Notes

A MODIFICATION OF MASSON'S TETRACHROME STAIN

For Routine Paraffin Sections of Tissue Fixed in Solution of Formaldehyde and Saline Solution

CHARLES P. LARSON, M.D., C.M., TACOMA, WASH.
AND E. J. LEVIN, SOAP LAKE, WASH.

During the past year trichrome and tetrachrome stains have been adopted by many laboratories as routine stains for paraffin sections. This has been due largely to the wide acceptance of Goldner's [1] modification of the Masson [2] stain. The original Masson trichrome stain still gives the most precise cytoplasmic and nuclear detail, but because of the length of time required for staining, the special fixation and the necessity of handling sections individually, it cannot be adopted by most pathologists for routine work.

The modification described here allows for the use of tissue fixed in solution of formaldehyde–saline solution and is very similar to the method of Goldner, but in view of the superior results which we have secured with slight alterations of his technic we feel justified in publishing our routine. Without counting the time required for deparaffination, the staining of a slide with this technic requires sixty minutes. Trays of 30 or more slides may be stained at one time with no individual handling and with surprisingly uniform results.

This stain has proved to be exceptionally good for all routine surgical and autopsy material and has the following advantages not possessed by hematoxylin and eosin: It differentiates every muscle fiber from connective tissue; it differentiates nerve tissue; it gives unusually clear detail in neoplastic growths, and as a routine survey stain for the central nervous system it is eminently satisfactory, as it brings out neuronal details and demarcates demyelinations. However, a word of caution may be given to those unaccustomed to the use of trichrome stains: There may be difficulty in evaluating the degree and extent of simple exudative and granulomatous lesions. With a little experience in interpreting the stain this difficulty will be overcome.

The stain may also be used on material fixed in Zenker's solution, provided the usual routine following the use of this fixative is adhered to, i. e., with iodine and thiosulfate and steps 3 and 4 in the routine left out.

From the Tacoma General Hospital, Tacoma (Dr. Larson), and McKay Memorial Research Hospital, Soap Lake, Wash.

1. Goldner, J.: Am. J. Path. **14**:237, 1938.

2. Masson, P.: J. Tech. Methods **12**:75, 1929.

PROCEDURE

1. Deparaffinize in xylene.
2. Hydrate by bringing down through graded alcohols.
3. Mordant for ten minutes in 5 per cent potassium dichromate.
4. Wash in running tap water for ten minutes.
5. Stain ten to fifteen minutes in Hansen's trioxyhematein.
6. Wash five minutes in running tap water.
7. Wash one minute in 1 per cent glacial acetic acid.
8. Stain five minutes in ponceau-fuchsin stain.
9. Wash two minutes in 1 per cent glacial acetic acid.
10. Differentiate one minute in 2 per cent phosphotungstic acid.
11. Wash two minutes in 1 per cent glacial acetic acid.
12. Stain in light green mixture for five minutes.
13. Wash for three minutes in 1 per cent glacial acetic acid.
14. Dehydrate in two changes of 95 per cent alcohol.
15. Clear in carbolxylene.
16. Mount in Canada balsam or dammar.

FORMULAS FOR PREPARATION OF REAGENTS

1. Alcohols may be ethyl or isopropanol (anhydrous).
2. Hansen's trioxyhematein is prepared as follows:

   Solution A.: Dissolve 10 Gm. of ammonium-ferric alum and 1.4 Gm. of ammonium sulfate in 150 cc. of distilled water with heat.

   Solution B:. Dissolve 1.6 Gm. of hematoxylin in 75 cc. of distilled water with heat.

Thoroughly cool both solutions. Then pour solution A into solution B (never vice versa), stirring constantly. When the mixture turns violet, heat it over flame and test it on filter paper for a sepia or a brownish black color. Remove the solution from flame immediately and cool by immersing the beaker in cold water. If the stain shows an olive green color on the filter paper, it is overoxidized. Do not boil the stain longer than one minute.

Dilute the prepared stain with an equal amount of 1 per cent sulfuric acid. Store in a stoppered bottle filled to the constricted portion, which exposes only a small area to oxidization. Filter each time before use.

The stain is stable for from four to six weeks.

3. The ponceau-fuchsin stain is made up as follows:

   Stock: Dissolve 1.5 Gm. of ponceau de xylidine and 0.5 Gm. of acid fuchsin in 200 cc. of 1 per cent glacial acetic acid.

   Staining solution: 10 cc. of stock and 2 cc. of 0.5 per cent aqueous azophloxin mixed with 88 cc. of 1 per cent glacial acetic acid.

4. Light green is prepared as follows:

   Stock: Dissolve 0.2 Gm. of light green S. F. in 100 cc. of 1 per cent glacial acetic acid solution.

   Staining solution: 20 cc. of stock to 80 cc. of 1 per cent glacial acetic acid solution.

5. Carbolxylene is made up of 3 parts xylene to 1 part chemically pure phenol.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—In recognition of his work in the field of cancer research, James B. Murphy, a member of the Rockefeller Institute for Medical Research, has received the decoration of Officer of the Order of Leopold from the king of Belgium.

Baxter L. Crawford, assistant professor of pathology in Jefferson Medical College, died Jan. 3, 1940, 53 years old.

In New York Medical College, L. Corsan Reid has been promoted to the position of associate professor, and F. D. Spear to that of assistant professor of pathology.

Francis Carter Wood, director of the Institute of Cancer Research of Columbia University, has been awarded the gold medal of the Radiological Society of North America in recognition of his achievements in the science of radiology.

Oran I. Cutler, professor of pathology in the College of Medical Evangelists, Loma Linda, Los Angeles, died as the result of an accident.

Fellowship in Pathology.—A research fellowship in the laboratory of pathology at the Collis P. Huntington Memorial Hospital, Boston, and in the department of pathology at the Harvard Medical School will be available September 1. According to *Science,* it carries a stipend of $3,000 and may be renewed for a second year. The fellow will be expected to devote most of his time to histologic and cytologic studies of the effects of radiation of different types on normal and pathologic tissue. Applications should be addressed to Dr. Shields Warren at the Collis P. Huntington Memorial Hospital, Boston.

Society News.—The American Association for the Study of Goiter will hold its next meeting at Rochester, Minn., April 15, 16 and 17, 1940. The Van Meter Prize Award for the best essay on original work on thyroid problems will be made at this meeting.

Maurice N. Richter has been elected president of the New York Pathological Society; Jean Oliver, vice president, and D. Murray Angevine, secretary.

A. F. Blakeslee, of the Carnegie Institution of Washington, has been elected president, and Paul R. Cannon, of the University of Chicago, vice president and chairman, of the section on medical sciences of the American Association for the Advancement of Science.

The next annual meeting of the American Society of Clinical Pathologists will be held at the Biltmore Hotel, New York, June 7, 8 and 9, 1940.

The annual meeting of the American Association of Pathologists and Bacteriologists will be held at the Mellon Institute of Industrial Research, Pittsburgh, March 21 and 22, 1940. A symposium will be held on the pathologic aspects of vitamin deficiencies.

The Pathological Society of Philadelphia has elected the following officers: president, Jefferson H. Clark; vice president, R. Philip Custer, and secretary-treasurer, Herbert L. Ratcliffe.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Histologic Changes in the Peripheral Nerves of the Rat in Vitamin B_1 Deficiency. C. O. Prickett, W. D. Salmon and G. A. Schrader, Am. J. Path. **15**:251, 1939.

The histologic changes in the peripheral nerves of rats suffering from either acute or chronic vitamin B_1 deficiency have been studied by the polarized light method and the sudan III method. The nerves of rats that had symptoms of acute deficiency showed only a few fibers with wallerian degeneration, such as may be seen in normal nerves, and mild edema. The nerves of control rats, which received adequate amounts of vitamin B_1 but with the food intake limited to that of the deficient rats, showed mild edema and more wallerian degeneration than was found in the nerves of the deficient rats. Nerves of deficient rats that became moribund without the development of typical neuromuscular symptoms showed more wallerian degeneration than those in which neuromuscular symptoms developed, but less than the control rats receiving adequate vitamin B_1 with a limited food intake. Residual symptoms, consisting of disordered equilibration or paralysis, occurred in rats in which a state of chronic deficiency had been produced by the administration of small amounts of thiamin chloride after the development of neuromuscular symptoms. The chronic deficiency produced marked changes of an edematous type in the peripheral nerves. The fibers were enlarged, in some cases showing large bulbous areas along their course, and contained increased amounts of isotropic material; in the most severe cases some fibers were completely isotropic. These changes were demonstrable by the polarized light method but not by the sudan III method. The rats eventually reached a stage at which cures were no longer possible even by the administration of relatively large doses of thiamin chloride. The changes in the nerves indicated that this was the result of irreparable damage to the tissues.

From Authors' Summary.

Fat Tissue in Experimental Exophthalmos. G. K. Smelser, Am. J. Path. **15**:341, 1939.

The injection of an extract of the anterior lobe of the pituitary gland produced exophthalmos and marked edema of the orbital fat and connective tissue. Fat tissues in various parts of the body were affected by the injection of the anterior pituitary extract but to a lesser degree than that in the orbit. The difference in the degree of edema produced in the orbit and in other fat tissues may have been due to the difference in structure of the orbital fat tissue. The edematous material found in the orbit appeared to be identical in composition with that found elsewhere in the same animal.

From Author's Summary.

Renal Function and the Number of Glomeruli in the Human Kidney. J. M. Hayman Jr., J. W. Martin Jr. and M. Miller, Arch. Int. Med. **64**:69, 1939.

The clearance of creatinine and urea, the maximum specific gravity of the urine and the blood pressure were correlated with the number of glomeruli per kidney (estimated after postmortem perfusion) for patients with and for patients without disease of the kidneys. In those with chronic glomerulonephritis and

nephrosclerosis the creatinine and urea clearance were closely correlated with the number of glomeruli. The maximum specific gravity of the urine falls with decrease in the number of glomeruli until the latter reaches 700,000 to 800,000 per kidney, after which it remains fixed in spite of further reduction in the number of glomeruli. If the number of glomeruli per kidney is less than 700,000, the systolic blood pressure is invariably above 150 mm. In certain patients with acute infections and jaundice the urea and creatinine clearance and the concentrating ability were both markedly reduced in spite of a normal number of glomeruli showing no significant change in histologic sections. FROM AUTHORS' SUMMARY.

ETIOLOGIC FACTORS IN EXPERIMENTALLY PRODUCED PONTILE HEMORRHAGES. L. V. DILL and C. E. ISENHOUR, Arch. Neurol. & Psychiat. **41**:1146, 1939.

Space-consuming intracranial lesions are occasionally associated with hemorrhages in the pons. In the authors' case, for instance, a subdural hematoma and a hemorrhage in the island of Reil were combined with multiple fresh hemorrhages in the pons. The authors see the cause of the pontile hemorrhages in the pressure exerted by the subdural hematoma on the brain and the forcing down (herniation) of the pons into the foramen magnum, which caused disruption of the parenchyma. They were able to produce pontile hemorrhages in dogs by increasing the intracranial pressure (by repeated inflation of a rubber balloon placed over the animal's parietal cortex). Hemorrhages (perivenous and periarterial) were produced in the pons, medulla and occasionally in the cerebellum and basal ganglions. Anoxemia as an etiologic factor was ruled out. GEORGE B. HASSIN.

EXPERIMENTAL HYPERTENSION BY CONSTRICTION OF THE AORTA. H. GOLDBLATT, J. R. KAHN and R. F. HANZAL, J. Exper. Med. **69**:649, 1939.

Constriction of the abdominal aorta just above the site of origin of both main renal arteries has little or no immediate effect on the blood pressure above the site of the clamp (carotid systolic or mean pressure), but after about twenty-four hours hypertension develops. Below the site of the clamp the immediate effect is a lowering of the femoral mean pressure. As the carotid systolic pressure becomes elevated, the femoral mean pressure also begins to rise and in some animals eventually reaches a level higher than normal, despite great constriction or even occlusion of the abdominal aorta. Constriction of the aorta just below the origin of both main renal arteries has no significant effect on the blood pressure (carotid systolic or mean pressure) above the site of the clamp. Below the site of the clamp the blood pressure falls and tends to remain down or at most returns only to the preoperative level. The uremic, convulsive (malignant or eclamptic) phase of hypertension, with renal excretory insufficiency and degenerative, necrotizing and inflammatory arteriolar lesions in many organs, has been produced by suddenly constricting to a great degree the abdominal aorta just above the origin of both main renal arteries. The renal excretory insufficiency in the animals in which hypertension develops is directly dependent on the degree of constriction of the abdominal aorta and especially on the rapidity with which constriction is produced. Hypertension following the constriction of the abdominal aorta just above the origin of both main renal arteries, whether or not accompanied by renal excretory insufficiency, is of renal origin. FROM AUTHORS' SUMMARY.

CATAPHORETIC EXPERIMENTS WITH SENSITIZED ERYTHROCYTES. C. G. ANDERSON and T. J. MACKIE, Brit. J. Exper. Path. **20**:270, 1939.

Anderson and Mackie have confirmed again the hemolytic effect of colloidal silicic acid and tannic acid.

EXPERIMENTAL ACUTE PERCHLORIDE INTOXICATION. G. L. MONTGOMERY, Brit. J. Exper. Path. **20**:316, 1939.

Montgomery studied the immediate effect of small intravenous doses of mercuric chloride in rabbits and found that, although there was no microscopic evidence of renal damage in the two hours of the experimental period, the injections produced circulatory collapse and proteinuria with a fall in the blood serum protein within fifteen minutes. The proteinuria quantitatively always exceeded the loss of serum protein, and the results suggest that the latter is replaced continuously from the tissue depots. Intravenously injected saline solution had no effect on the shock syndrome.

CARBON TETRACHLORIDE CIRRHOSIS OF THE LIVER. E. G. WHITE, J. Path. & Bact. **49**:95, 1939.

In pigs weighing 7 to 17 Kg. and aged 25 to 66 days the subcutaneous injection of a single dose of carbon tetrachloride of 0.6 cc. per kilogram of body weight seldom caused clinical disturbances, yet there was necrosis of the central half of each hepatic lobule. Repair was complete by the end of a week. Some animals, however, will not tolerate this dose, and an amount as small as 0.2 cc. per kilogram has caused death within twelve hours. The histologic changes occurring in the liver at intervals after a single dose are described. Repeated administration of carbon tetrachloride at four to five day intervals leads to cirrhosis which is characterized by a subdivision of hepatic lobules into small groups of liver cells ("pseudolobules") by collagen and precollagenous reticulum. The genesis of the process has been studied by examining samples of liver removed at intervals and finally by carrying out an autopsy. The most severe cirrhosis resulted from the heaviest dose, thirty-six injections of 0.2 cc. per kilogram of body weight; less severe changes were produced by twenty-eight doses of 0.1 cc. per kilogram or twenty-six doses of 0.05 cc. per kilogram. There was no ascites, icterus or splenomegaly. The experimentally produced cirrhosis appears to resemble the naturally occurring lesion in the pig. FROM AUTHOR'S SUMMARY.

RELATION OF THROMBOSIS, EMBOLISM AND APOPLEXY TO THE WEATHER. P. H. KAYSER, Virchows Arch. f. path. Anat. **302**:210, 1938.

In 253 cases of thrombosis, fulminant embolism and apoplexy, in which the condition was identified at autopsy, the onset of the vascular accident was correlated with the weather conditions at the time. Kayser concludes that his material reveals a relation between the state of the weather and the onset of thrombosis and apoplexy, these diseases, which he terms meteorotropic, occurring more often during warm fronts than during cold fronts. Thrombosis, embolism and apoplexy do not occur during periods of uniform weather; during such periods deaths from carcinoma and infectious diseases are more numerous. A seasonal variation of the vascular diseases studied was not observed. In regions with a continental climate thrombosis is rarely seen. Patients susceptible to meteorologic environment react abnormally to every change in the weather. The electronic effect of the atmosphere is held to be the most active factor in the action of weather; such an effect, working through the skin on the vegetative nervous system, causes slowing of the circulation, which favors thrombosis. Petersen's monumental work on meteorologic pathologic changes is not included in the bibliography of 31 titles.

O. T. SCHULTZ.

EFFECT OF TRANSPLANTATION OF LIVER INTO MICE PREVIOUSLY TREATED WITH LIVER. A. SYMEONIDIS, Virchows Arch. f. path. Anat. **302**:443, 1938.

The subcutaneous homologous transplantation of liver into mice previously given injections of an emulsion of liver was followed by a more intense and more

rapidly developing inflammatory reaction than was observed in the controls. Encapsulation of the implant was more rapid, and necrosis more complete. The surrounding granulation tissue was less highly vascularized than in the controls.

O. T. SCHULTZ.

Pathologic Anatomy

THE LYMPHOID NODULES OF HUMAN BONE MARROW. R. J. WILLIAMS, Am. J. Path. **15**:377, 1939.

No pathologic significance can be attached to the presence in the marrow of lymphoid nodules of the type, size and number described in this report. The evidence appears to justify the concept that the lymphoid nodules are essentially normal, though perhaps variable, constituents of the active red marrow of adults.

FROM AUTHOR'S SUMMARY.

CARDIAC SEQUELAE OF EMBOLISM OF THE PULMONARY ARTERY. H. HORN, S. DACK and C. K. FRIEDBERG, Arch. Int. Med. **64**:296, 1939.

A group of 42 cases of embolism of the pulmonary artery has been studied, in 8 of which recent structural changes in the myocardium ordinarily resulting from acute myocardial ischemia were revealed. The factors necessary for the production of such myocardial changes are discussed. These are shock, asphyxia and exaggerated vagal reflexes resulting from obstruction of the pulmonary arteries. These factors, alone or in association, lead to insufficiency of the coronary circulation.

Morphologic evidence of coronary insufficiency in cases of embolism of the pulmonary artery is more likely to occur if there are recurrent embolization, narrowing of the coronary arteries, cardiac hypertrophy and adequate duration of life after embolism. Anatomic changes in the myocardium in persons with embolism of the pulmonary artery may be considered the end result of the myocardial ischemia which accounts for the characteristic electrocardiographic changes. The resemblance of electrocardiographic changes in cases of embolism of the pulmonary artery to those in cases of myocardial infarction of the posterior wall may be explained by the diminished flow through the right coronary artery resulting from increased tension in the right ventricle.

FROM AUTHORS' SUMMARY.

OCCURRENCE AND DISTRIBUTION OF CALCIFIED PLAQUES IN THE SPINAL ARACHNOID IN MAN. E. Y. HERREN, Arch. Neurol. & Psychiat. **41**:1180, 1939.

The cells of the spinal arachnoid membrane often form whorls and become calcified, producing so-called plaques. Weed considered the plaques a manifestation of advanced age, with which opinion Herren does not agree. Herren studied the distribution and location of the plaques in human beings of various ages. He found the largest number of plaques in the dorsolumbar and the lumbosacral areas, and especially on the posterior surface of the cord. Persons without plaques were on the average eight and eight-tenths years older than those with plaques. The plaques form by deposition of calcium in the whorls of the arachnoid cells, but why they should arise with preference in certain areas, Herren could not explain.

GEORGE B. HASSIN.

CHRONIC ULCERATIVE CECITIS IN THE RAT. B. F. JONES and H. L. STEWART, Pub. Health Rep. **54**:172, 1939.

A brief description is presented of a spontaneous disease of rats characterized by chronic ulcerative cecitis and chronic lymphangitis, lymphedema and lymphoid hyperplasia of the lymph nodes of the mesentery.

FROM AUTHORS' SUMMARY.

CYCLIC CHANGES IN THE CHROMATIN OF THE NUCLEI OF THE ENDOMETRIUM. R. CLEVELAND, Surg., Gynec. & Obst. **69**:18, 1939.

A study of 200 specimens of human endometrium obtained from normal women and from others exhibiting various degrees of ovarian failure revealed that two forms of nuclei can be distinguished, namely, a granular and a nongranular, or solid, homogeneous, form. Further, the granular form of nucleus showed two distinct types of distribution of the chromatin, aggregate and diffuse. The former type of distribution appeared almost uniformly in the nuclei of endometrium presenting the characteristics of proliferation. It was not predominant in the secretory or menstrual phases. It appears, then, that the aggregate type of distribution of the chromatin is characteristic of human endometrium which is under the influence of the follicular hormone alone either normally or as a manifestation of second degree ovarian failure. As a whole, tissue from women with first degree ovarian failure showed the aggregate type of chromatin in the stroma more frequently than did tissue obtained from normal women during the secretory and menstrual periods. On the other hand, the gland chromatin in endometrium associated with first degree ovarian failure showed no significant differences from normal. These observations suggest that differences in the threshold response of the gland and stromal nuclei of the human endometrium to hormonal stimulation may furnish the basis for determining fluctuations in endocrine levels.

FROM THE AUTHOR'S SUMMARY (WARREN C. HUNTER).

HISTOLOGY OF FREI'S REACTION. F. FRANCHI, Gior. ital. di dermat. e sif. **80**:369, 1939.

The Frei intradermal reaction in the presence of venereal lymphogranuloma has a tuberculoid structure in which epithelioid cells and giant cells of the Langhans type are prominent elements. In the presence of venereal lymphogranuloma the reaction to the Frei antigen is specific; the reactions to bacterial vaccines are nonspecific and of simple inflammatory nature.

CHANGES IN THE UMBILICAL ARTERY. G. SCHALLOCK, Virchows Arch. f. path. Anat. **302**:195, 1938.

The umbilical artery was studied histologically during the fetal period and at varying periods after birth. Ligation of the cord after birth leads to immediate contraction of the vessel, with swelling of the subendothelial ground substance. Contraction is followed by dilatation, during which the ground substance increases and histolytic changes occur. The further changes consist of necrosis of the wall with or without thrombosis, arteriosclerosis with or without obliteration of the lumen, hyperplasia especially of the elastic tissue, and proliferative changes with formation of a new lumen. These changes are not due to increased pressure in the artery but are an adaptation to the functional alterations that follow ligation of the cord. The escape of blood or serum into the wall of the vessel is an etiologic factor in the later changes.

O. T. SCHULTZ.

INVOLVEMENT OF BONE IN LEUKEMIA. K. APITZ, Virchows Arch. f. path. Anat. **302**:301, 1938.

Apitz presents a detailed histologic description of the skeletal changes in 2 cases of lymphoid leukemia and describes three types of involvement. The least frequent manifestation of the disease is localized erosion of bone through lacunar resorption. This is looked on as evidence of an invasive or neoplastic character of leukemic cells. Necrosis of the infiltrated marrow may lead to death of bone and to spontaneous fractures; this form of skeletal involvement is not due to aggressive growth of the leukemic tissue. Leukemic infiltration of the marrow may lead to osteoporosis and localized atrophy of bone with spontaneous fracture. In children leukemia may interfere with bone growth and may be associated with subperiosteal bone formation.

O. T. SCHULTZ.

Microbiology and Parasitology

MOOSE ENCEPHALITIS. L. S. KING, Am. J. Path. **15**:445, 1939.

A subacute or chronic leukencephalitis occurring naturally in moose is described. The characteristic picture consists of a mild degree of perivascular demyelination, with formation of neutral fat and with fibrous gliosis disproportionate in extent to the loss of myelin. There may be mild inflammation restricted to the white matter. There is evidence suggestive that a primary inflammatory reaction involving gray matter observed in 1 of 8 animals may represent a separate condition. Attempted animal passage of fresh material from an infected moose was unsuccessful. The cause of this leukoencephalitis is obscure, but various possibilities are discussed.

FROM AUTHOR'S SUMMARY.

PNEUMONIA PRODUCED BY TYPHUS RICKETTSIAE. M. R. CASTANEDA, Am. J. Path. **15**:467, 1939.

The intranasal inoculation of mice and rats with typhus virus (orchitic variety) has given rise to hemorrhagic lesions of the lungs which kill the mice in ninety-six hours and the rats in seventy-two hours. The lungs show, in sections and smears, considerable numbers of rickettsia bodies, which have been obtained in pure suspension by grinding and fractional centrifugation. Rabbits have been infected by the intratracheal route with or without forcing down the body temperature. Hemorrhagic pneumonia develops, and rickettsias are present in large numbers in smears and in sections of the lungs, but the animals subjected to a low body temperature present greater quantities of rickettsia bodies. These rabbits die in from forty-eight to ninety-six hours after inoculation. The rabbits not submitted to a low body temperature die after a longer period of time and show lesions of the lungs which are more extensive but which contain fewer rickettsias. To produce massive infection of the lungs it is necessary to inoculate considerable numbers of rickettsias. This method of cultivating rickettsias has proved useful in obtaining typhus vaccine for practical purposes.

FROM AUTHOR'S SUMMARY.

DIABETES AND PULMONARY TUBERCULOSIS. H. F. ROOT and W. T. BLOOR, Am. Rev. Tuberc. **39**:714, 1939.

A study of the various etiologic factors in the pulmonary tuberculosis of 364 diabetic patients points to the disturbed nutrition of the diabetic patients as next in importance to their contact with patients with open tuberculous lesions. The incidence of pulmonary tuberculosis in adults with diabetes does not show a decrease corresponding with the general decrease in mortality from tuberculosis in the community. Pulmonary tuberculosis followed the onset of diabetes in 83 per cent of the cases. In 126 autopsies on diabetic patients with pulmonary tuberculosis it was found that the outstanding features were many healed foci, a tendency toward the formation of tough, fibrous pleural adhesions and a high frequency of caseating lesions with cavitation. On chemical analysis the lungs of these patients showed strikingly lower concentrations of phospholipid and lipid than the lungs of nondiabetic patients. The advantages of protamine zinc insulin in the treatment of tuberculous diabetic patients are discussed, and dietary information is given. Diabetic patients make excellent subjects for pneumothorax and thoracoplasty. The prognosis for the diabetic patient with pulmonary tuberculosis has been greatly improved by insulin and can be further enhanced by early diagnosis. Routine roentgen examination of every diabetic person's chest is recommended.

H. J. CORPER.

ORAL TUBERCULOSIS. J. C. BRYANT, Am. Rev. Tuberc. **39**:738, 1939.

Tuberculous involvement of the oral cavity is comparatively rare; only 17 cases have been detected in some 7,000 cases of far advanced tuberculosis over a period of eighteen years. Tuberculous lesions of the tongue frequently have a history of mechanical irritation from the sharp edges of decayed and abraded teeth, broken silver fillings, gold inlays or crowns, or broken artificial teeth. These sharp edges traumatize the tissue. The constant irrigation and bathing of the oral tissues by the salivary and mucous secretions render the oral tissues highly resistant to tuberculous infection. Tuberculosis of the oral cavity is a secondary manifestation of a far advanced pulmonary condition with an unfavorable prognosis. When the prognosis is favorable, tuberculous lesions are seldom formed in tooth sockets following extractions, despite the presence of sputum heavily laden with the bacilli.

H. J. CORPER.

PATHOGENICITY OF AVIRULENT PNEUMOCOCCI FOR ANIMALS DEPRIVED OF LEUKOCYTES. A. R. RICH and C. M. MCKEE, Bull. Johns Hopkins Hosp. **64**:434, 1939.

When nonencapsulated pneumococci, which are nonpathogenic (avirulent) for normal rabbits, were introduced into the tissues of rabbits deprived of leukocytes, they exhibited the qualities of virulent pneumococci; i. e., they multiplied to enormous numbers and produced a progressive local infection, leading in one third of the animals to septicemia. Recovered from the blood at the death of the animal, the organisms were still nonencapsulated and avirulent for rabbits possessing leukocytes. Since in the leukopenic animal the avirulent pneumococcus behaves as a virulent pathogen, the transformation of the virulent, encapsulated form into the avirulent, nonencapsulated form entails no change which prevents its survival and free proliferation in the tissues if it is unmolested by phagocytes. The local lesion produced by the nonencapsulated, avirulent pneumococcus in the leukopenic animal exhibits the hemorrhages and necrosis of tissue familiar in the lesions produced by the encapsulated, virulent pneumococcus in the normal animal. The factor responsible for virulence (the polysaccharide capsule) is therefore not necessary for the production of the characteristic type of tissue damage that virulent pneumococci produce. The power to damage the tissues so resides in the somatic rather than in the capsular portion of the bacterium, and the change in the organism resulting in the failure to produce a capsule does not alter the ability of the somatic portion to produce the characteristic lesion.

FROM AUTHORS' SUMMARY.

MENINGOCOCCUS INFECTION OF CHICK EMBRYO. G. J. BUDDINGH and A. D. POLK, J. Exper. Med. **70**:485, 499 and 511, 1939.

It has been found that the meningitis in the chick embryo following inoculation of meningococci into the body wall or into the amniotic cavity is due to invasion by way of the blood stream only.

STUDIES ON EASTERN EQUINE ENCEPHALOMYELITIS. L. S. KING, J. Exper. Med. **70**:675 and 691, 1939.

Genesis of the Disease in the Guinea Pig.—The virus of Eastern equine encephalomyelitis when injected peripherally into the guinea pig invades the blood stream and passes directly from the blood stream into the brain. This seems to be the principal, though not necessarily the exclusive, route of infection. Once the virus is in the nervous system, its further spread may occasionally be determined by anatomic connections.

Intraocular Infection with Fixed Virus in the Guinea Pig.—The behavior of a fixed strain of Eastern equine encephalomyelitis virus was studied in guinea pigs after intraocular inoculation. Such inoculation concerns the central and not the

peripheral nervous system. The susceptibility to the intraocular injection lies midway between that to the highly effective intracerebral and that to the ineffective peripheral injection. The virus must act for ten to thirteen hours in order to induce a fatal infection. Removal of the inoculated eyeball before the expiration of this interval almost always prevents fatality, although it may allow immunity to develop. At suitable intervals after injection of the virus into the eye, it may be recovered from successive and appropriate optic centers before it is demonstrable in nonoptic portions. Approximately twenty-four hours are required for the virus to reach a significant concentration in the contralateral geniculate body; thirty-six hours, in the contralateral visual cortex. Significant amounts of virus may be present in the optic chiasm and tract prior to involvement of the higher centers. Virus placed in contact with the retina produces an insignificant, essentially nonspecific reaction as compared with that produced at the site of direct intracerebral inoculation. In the retina there is no necrosis of ganglion cells unless there is a complicating intraocular infection. In the cerebral visual centers the first reaction is inflammatory and interstitial, and it may appear in the lateral geniculate body as early as twenty-four hours after injection of the virus. Neuronal necrosis is not the primary result of the action of the virus on the nervous system in these experiments. The distribution of lesions in the brain is in excellent agreement with the method of direct testing for virus content, and is far more accurate than the latter. The virus in its primary distribution through the nervous system follows the nerve pathways of the optic system. This occurs within the central nervous system, where presumably there is first an involvement of the body of the nerve cell and then a spread along the cell process or axon.

FROM AUTHOR'S SUMMARIES.

INTER-RELATIONSHIPS BETWEEN AMINO ACIDS IN THE NUTRITION OF BACILLUS ANTHRACIS. G. P. GLADSTONE, Brit. J. Exper. Path. **20**:189, 1939.

Gladstone grows Bacillus anthracis in a chemically defined amino acid medium. When certain amino acids are omitted singly, growth ceases, and the omitted amino acid thus appears to be indispensable. However, if in addition another particular amino acid is omitted, growth occurs, showing that the first amino acid omitted is not indispensable. It appears that each amino acid is really inhibitory but that when both are present they are stimulative. These observations may have a bearing on conclusions drawn from nutritional studies in animals.

EFFECT OF FOREIGN TISSUE EXTRACTS ON THE EFFICACY OF INFLUENZA VIRUS VACCINES. D. H. ANDREWES and W. SMITH, Brit. J. Exper. Path. **20**:305, 1939.

Andrewes and Smith have attempted to analyze the factors which determine the efficacy of influenza virus vaccines. The detrimental effect of tissue extracts from foreign animal species, especially when inactivated vaccines are employed, makes one fear difficulties in immunizing man satisfactorily. It is suggested, however, by analogy in work on ferrets, that the practical difficulties may be less if one attempts only to reenforce the basic immunity which most human adults possess against influenza.

CHEMOTHERAPY OF EXPERIMENTAL PNEUMONIA. A. VAISMAN, LEVADITI and D. KRASSNOFF, Ann. Inst. Pasteur **62**:36, 1939.

Vaisman, Levaditi and Krassnoff develop the thesis that sulfanilamide and various related compounds (or derivatives thereof produced by the infecting organisms) act primarily by interfering with an increase in capsular substance, with the result that phagocytosis is facilitated. This makes the therapeutic role of these substances a "natural" cure. The thesis is compatible with the observation that organisms are not always killed and that the beneficial results are due to the reactions of the body cells.

M. S. MARSHALL.

Immunology

PROTECTIVE ANTIBODIES IN THE SERUM OF SYPHILITIC RABBITS. T. B. TURNER, J. Exper. Med. **69**:867, 1939.

During the course of syphilitic infection in rabbits, specific humoral antibodies develop which can be demonstrated by an appropriate "protection test." The presence of these antibodies is associated with a high degree of acquired immunity to the disease. FROM AUTHOR'S CONCLUSION.

CELLULAR REACTIONS TO A DYE PROTEIN. F. R. SABIN, J. Exper. Med. **70**:67, 1939.

The use of an antigen which can be seen within cells demonstrates that one may stimulate the phagocytic cells either of the liver and spleen or of the tissues and lymph nodes to produce antibodies. The appearance of antibodies in the serum correlates with the time when the dye protein is no longer visible within the cells and with the phenomenon of a partial shedding of their surface films. It is thus inferred that the cells of the reticuloendothelial system normally produce globulin and that antibody globulin represents the synthesis of a new kind of protein under the influence of an antigen. An antigen is a substance which can specifically modify the synthesis of the cytoplasm of the cells of the reticuloendothelial system. FROM AUTHOR'S CONCLUSIONS.

BLOOD GROUPS AND MN-TYPES OF ESKIMOS IN EAST GREENLAND. V. FABRICIUS-HANSEN, J. Immunol. **36**:523, 1939.

In a study of 569 pure Eskimos in the eastern part of Greenland (Angmagssalik district) the percentile frequencies of blood groups O, A, B and AB were 23.9, 56.2, 11.2 and 8.7, respectively, and the frequencies of blood types M, MN and N were 83.48, 15.64 and 0.88, respectively. This is the first study of the distribution of the blood type factors M and N in Eskimos. The values of the blood group factors A, B and O differ from those previously reported by showing a lower frequency of O and a higher frequency of B. I. DAVIDSOHN.

IMMUNE REACTIONS OF VIRUS MYXOMATOSUM. R. E. HOFFSTADT and K. S. PILCHER, J. Infect. Dis. **65**:103, 1939.

Elementary bodies of myxomatosum virus similar to those found in rabbit tissue were found in the chorioallantoic membranes of infected developing chick embryos. The soluble antigen associated with myxomatosum virus in tissues of infected rabbits was found in the infected chorioallantoic membranes of chick embryos after prolonged serial passage. FROM AUTHORS' SUMMARY.

INCIDENCE OF PROSTATIC CARCINOMA. E. P. GAYNOR, Virchows Arch. f. path. Anat. **301**:602, 1938.

In 1,000 consecutive, unselected necropsies on men aged 40 years and over, the entire prostate was removed and fixed in 10 per cent solution of formaldehyde. The prostates of 40 men aged 25 to 40 years were added to the material. The material was worked up by the razor section technic of Terry. Each prostate entire was cut into slices 2 to 3 mm. thick; these were stained with toluidine blue and examined at magnifications of 20 to 30 diameters. The anatomic division of the prostate into five lobes, posterior, middle, two lateral and anterior, was accepted, although the middle and anterior lobes often consisted of only a few glandular structures. Carcinoma was detected in 191 prostates, a percental incidence of 18.4. The incidence increased progressively with age, from 10.4 per cent in the sixth decade to 40 per cent in the tenth. In 162 prostates the tumor did not involve an entire lobe and was not macroscopically visible; in this group

multiple tumors were observed (203 carcinomas in 162 prostates). The posterior lobe was most frequently involved (60 per cent); the middle lobe, very seldom. The earliest localization was in the peripheral portion of the gland, and the inner fibromuscular capsule was frequently invaded. The outer fibrous capsule was rarely penetrated, and growth outside the gland was rarely observed, although growth into the blood vessels and lymphatics was frequently observed. Benign hypertrophy did not appear to be a factor in the development of carcinoma; the latter was relatively no more frequent in hypertrophied prostates than in those of normal size. Atrophy also did not appear to be a factor. Secondary carcinoma of the prostate was observed only twice.

O. T. SCHULTZ.

IMMUNOLOGIC RELATIONSHIP OF SHOPE'S RABBIT FIBROMA VIRUS TO THE VIRUS OF INFECTIOUS MYXOMATOSIS. C. E. VAN ROOYEN and A. J. RHODES, Zentralbl. f. Bakt. (Abt. 1) **142**:149, 1938.

Specific complement-fixing antibodies have been demonstrated in the serum of rabbits immunized with myxoma virus. Serum of rabbits immunized against Shope's virus contains complement-fixing antibodies for that agent but not for myxoma virus. After complement-fixing antibodies have disappeared from the serum of rabbits immunized to Shope's virus and the animals have been inoculated with myxoma virus, complement-fixing properties specific for the latter appear. Complement-fixing antibodies for myxoma virus occurring in the serum of immune rabbits are usually present for at least eight weeks. Similar properties for Shope's virus generally disappear before this time. The results of these tests evidence no serologic relationship between the two viruses.

PAUL R. CANNON.

Tumors

EFFECT OF FREEZING IN VITRO ON SOME TRANSPLANTABLE MAMMALIAN TUMORS AND ON NORMAL RAT SKIN. G. B. MIDER and J. J. MORTON, Am. J. Cancer **35**:502, 1939.

After exposure to —74 C. in vitro, Walker rat carcinoma 256 and mouse sarcomas 180 and 37 grew on subcutaneous transplantation. The effects of the rate of freezing, the duration of the frozen state (up to twenty-four hours), the number of repeated freezings and thawings, and the physical state of the tumor are discussed. The squamous epithelial and connective tissue cells of normal adult rat skin may grow after a single freezing to —74 C.

FROM AUTHORS' SUMMARY.

PRODUCTION OF LUNG TUMORS IN MICE. M. B. SHIMKIN, Am. J. Cancer **35**:538, 1939.

Mice weighing about 30 Gm. tolerated an intratracheal injection of 0.1 cc. of water, saline or serum suspension; the mortality from the procedure was about 35 per cent. Primary pulmonary tumors occurred in over 90 per cent of strain A mice within four months after intratracheal introduction of 0.1 mg. of 1,2,5,6-dibenzanthracene or methylcholanthrene dispersed in 0.1 cc. of horse serum and cholesterol. The intratracheal route of administration is not as convenient or as efficacious as the intravenous.

FROM AUTHOR'S SUMMARY.

VITAMIN E AND EXPERIMENTAL TUMORS. C. CARRUTHERS, Am. J. Cancer **35**: 546, 1939.

Two pure strains of mice, A and C57, have been used in a study of the possible effects of dietary content of vitamin E on the incidence and metastasis of tumors induced by methylcholanthrene. The extent and the frequency of spontaneous mammary tumors in the females of susceptible strain A have also been examined.

Synthetic diets containing the usual constituents—casein, lard, starch, salts—and adequate supplies of the vitamins except E proved adequate for growth even when they contained rancid lard, which destroys even traces of vitamin E. Supplemented with vitamin E concentrate, prepared from cottonseed oil, these diets are also adequate for reproduction but not for lactation. The administration of vitamin E concentrate had no significant effect on the carcinogenic action of methylcholanthrene dissolved in lard or spermaceti and injected subcutaneously. When spermaceti was used as a solvent, tumors arose, on the average, twenty and six-tenths days earlier in strain A mice. Conclusions regarding the effect of nutritional factors on carcinogenesis induced by potent cancer-producing hydrocarbons must be drawn with care; the vigorous action of these carcinogens seems to be independent of the nutritive state of the animal. The etiologic nature of tumors induced by methylcholanthrene has been rendered questionable by the fact that many animals, approximately 30 per cent, acquired epidermoid carcinomas; most other investigators have reported sarcomas. The hydrocarbon produces more extensive ulceration in strain A than in strain C57. In both strains the tumors invaded the musculature, 58 per cent of them when the hydrocarbon was dissolved in spermaceti, 82 per cent of them when it was disolved in lard. In strain A 41 per cent of the animals had nonmetastatic "lung tumors" when spermaceti was used as solvent of the carcinogen, 45 per cent when lard was employed. Methylcholanthrene thus markedly increases the incidence of these tumors which are hereditary characteristic of strain A. Spontaneous mammary carcinomas arose in strain A females whose vitamin E stores permitted a "first litter" fertility. On the rancid diet the incidence of mammary tumors was much lower. Whether this was due to lack of vitamin E or to some other dietary influence has not yet been determined.

FROM AUTHOR'S SUMMARY.

THE STATISTICAL RELATION BETWEEN GOITER AND CANCER. J. F. MCCLENDON, Am. J. Cancer **35**:554, 1939.

Although the statistics so far studied may not be regarded as entirely sufficient to establish a relation between goiter and cancer, the evidence is pretty conclusive that thyroid adenoma may predispose to cancer of the thyroid, and the evidence is ample to act as a warning that goiter may increase the cancer rate. Therefore prophylaxis against goiter by administration of iodine to the young and administration of desiccated thyroid to the aged may be a precaution worth taking to prevent an increase in the incidence of cancer.

PAINFUL SUBCUTANEOUS TUBERCLE (TUBERCULUM DOLOROSUM). A. P. STOUT, Am. J. Cancer **36**:25, 1939.

An investigation of 2,081 superficial tumors of skin and subcutaneous tissues showed that 20, or approximately 1 per cent, were associated with attacks of paroxysmal pain. The types of tumor included not only leiomyoma and glomus tumor but also neurofibroma, fibroma, fibrosarcoma, keloid, dermoid cyst and benign epithelioma in a sebaceous cyst. The tuberculum dolorosum, therefore, is not confined to a single form of tumor but may manifest itself in a variety of morphologic types. No adequate explanation for the occurrence of the attacks of paroxysmal pain could be found.

FROM AUTHOR'S SUMMARY.

TRANSMISSIBLE MONOCYTOMA OF THE MOUSE. M. R. LEWIS, Am. J. Cancer **36**:34, 1939.

The strain-specific transplantable growth designated as monocytoma no. 255, which arose from the implantation of a white spot on the spleen of a mouse which had received an intraperitoneal injection of dibenzanthracene four hundred and eleven days previously, is composed of malignant cells of the monocyte type (the majority of them being permanently altered large epithelioid cells) and is transplantable by means of living cells of the same kind.

FROM AUTHOR'S CONCLUSION.

CANCER AND JEWS. G. WOLFF, Am. J. Hyg. (Sect. A) **29**:121, 1939.

These new statistics from Berlin seem to show that there is little real difference between Jews and non-Jews in the general frequency of death from malignant disease if the higher mortality of the youngest age groups is left out of consideration because it depends on small numbers and is opposed to the somewhat lower mortality of Jews in a majority of the other age groups. On the whole, the standardized mortality rate for the Jewish population is slightly less than that for the whole population. These differences tend to disappear and may well be due to changes of social and occupational structure, since mortality from cancer is clearly associated with some occupational differences and perhaps influenced to a certain degree by social factors; for example, opportunities for early diagnosis and treatment. Here, then, racial factors seem to play no part. On the other hand, there is plainly a difference between the two groups in the localizations of the disease. Whether this difference is a racial distinction or the consequence of a difference in habits of life remains obscure. The recent observations of Handley speak against any racial immunity from carcinoma of the uterus; they require confirmation before they can be fully accepted. In general, it seems improbable that in such a racial mixture as the religious community of the Jews must be from the anthropologic point of view there should be racial differences respecting the pathologic nature of malignant disease. It seems far more probable that in this matter habits and customs, eating and drinking, occupations and social status have an influence, limited but distinct, which has not yet been explained. It is true that cancer is distinct from the epidemic diseases, which show seasonal, secular and geographic variations, and is a reasonably constant cause of mortality, depending principally on age. It may be that a study of heredity will bring about clearer conclusions by tracing the course of events in particular "cancer families"—an important if difficult piece of work. This, however, is a problem totally different from that of racial ideology, which has but little in common with the exact study of inheritance.

FROM AUTHOR'S CONCLUSIONS.

PINEALOMA. A. H. BAGGENSTOSS and J. G. LOVE, Arch. Neurol. & Psychiat. **41**:1187, 1939.

Of 10 cases of tumor of the pineal body (pinealoma), the authors classified 2 as cases of glioma (spongioblastic pinealoma). In 5 cases the structure of the tumor resembled phases in the development of the normal pineal body. For instance, in 3 of these 5 cases the histologic picture suggested the pineal bodies of infants of about 2 and 9 months. In the remaining, the third, group of cases the authors included cases of so-called pineal ependymoma, which, they state, is differentiated from pure ependymoma by the presence of large cells possessing the characteristic features of pineal parenchymal cells. In short, in some cases a pineal tumor arises from parenchymal cells of the pineal body, and in some, from the neurologic cells.

GEORGE B. HASSIN.

FAMILIAL MAMMARY TUMORS IN THE RABBIT. H. S. N. GREENE, J. Exper. Med. **70**:147, 159 and 167, 1939.

The clinical histories of two different types of familial mammary cancer in the rabbit are given. As to one type, the first clinical sign of an abnormality of the breast was a sudden intense engorgement, after which the disorder passed through stages of cyst formation and benign neoplasia to cancer with metastasis. In regard to the second type, the neoplasia originated in clinically normal breast tissue, and there was no history of antecedent mammary abnormality.

The pathologic histories of two types of familial mammary cancer in the rabbit are given. One type was distinguished by characteristic antecedent mammary

changes similar to those found in Schimmelbusch's disease in women and by a distinctive papillary structure. The second type originated in normal breast tissue and was characterized histologically by an atypical proliferation of acini.

The clinical and the pathologic course of 25 mammary tumors in rabbits are described. The antecedent history of the breast and the morphologic character of the growths allowed a natural classification into two distinct types, one of which was distinguished by preexisting cystic mastitis and a papillary structure, while the other originated in clinically normal mammary tissue and was characterized by an adenomatous structure. The two types of neoplasia occurred almost exclusively in two family groups, and heredity played a fundamental role both in the occurrence of the tumors and in the determination of the type. Endocrine changes comparable with those found in animals after long-continued administration of estrogenic substances occurred in the tumor-bearing rabbits, and it was inferred that the spontaneous growths represented a natural counterpart of the experimental induction of neoplasia with estrone (theelin).

FROM AUTHOR'S SUMMARIES.

MANNER OF GROWTH OF FROG CARCINOMA. B. LUCKÉ and H. SCHLUMBERGER, J. Exper. Med. **70**:257, 1939.

The adenocarcinoma which commonly occurs in the kidney of the leopard frog has been transplanted into the anterior chamber of the eye, where the characteristics of its growth have been studied by direct observation with the slit lamp microscope. Such observations have been amplified by photographs taken at intervals as permanent and objective records of the mode of development and progress of the growths from earliest to advanced stages. FROM AUTHORS' SUMMARY.

A CONSIDERATION OF CERTAIN TYPES OF BENIGN TUMORS OF THE PLACENTA. A. A. MARCHETTI, Surg., Gynec. & Obst. **68**:733, 1939.

The comparative rarity of chorioangioma is attested by the 217 instances now on record in the literature. From a study of 8 cases Marchetti differentiates several types on the basis of histologic structure and pattern. The cellular or immature type, the vascular or more mature type and the type accompanied by varying degrees of degenerative changes may intermingle or show all gradations in the same tumor. It is fairly well established that the tumor tissue originates from the chorionic mesenchyme, with the proliferating endothelium and blood vessels playing the leading role, while the stroma has a subordinate part or is passive. It is still an open question whether the placental chorioangioma is a true tumor or a malformation. Chorioangioma is of little clinical significance.

WARREN C. HUNTER.

CARCINOGENIC AGENTS PRESENT IN THE ATMOSPHERE AND THE INCIDENCE OF PRIMARY LUNG TUMORS IN MICE. J. A. CAMPBELL, Brit. J. Exper. Path. **20**:122, 1939.

Campbell finds that dust from tarred roads and chimney soot contain carcinogenic agents. The former definitely increases the incidence of primary pulmonary tumors in mice; the latter produces little change. The road contains other agents (inorganic substances) which aid the tar in producing its effects. The carbon of the soot mitigates the effects of the tar in chimney soot.

A COMPARISON OF SOME CARCINOGENIC WITH NONCARCINOGENIC COMPOUNDS AS TO PHOTODYNAMIC ACTIVITY. I. DONIAC, Brit. J. Exper. Path. **20**:227, 1939.

Colloid suspensions of 3,4-benzpyrene in water sensitize paramecia to light in dilutions up to 1: 100,000,000. The sensitivity of the paramecia is markedly increased by contact with the colloid in the dark. The photo-oxidation products

of 3,4-benzpyrene are also photodynamic, but their action is not increased by contact in the dark. The carcinogenic hydrocarbons 1,2-benzanthracene, cholanthrene and methylcholanthrene have the same action on paramecia as 3,4-benzpyrene. The noncarcinogenic sensitizers to light acridine, acriflavine, eosin and quinine sulfate are less potent photodynamically than 3,4-benzpyrene, and their action is not increased by contact in the dark. The photodynamic property is a sensitive means of assaying biologically the aforementioned carcinogenic hydrocarbons.

FROM AUTHOR'S SUMMARY.

FIBROMA OF THE HEART. A. SYMEONIDIS and A. J. LINZBACH, Virchows Arch. f. path. Anat. **302**:383, 1938.

From the literature relating to so-called fibroma of the heart, a term which usually refers to an organized thrombus, the authors select 6 cases that have distinguishing characteristics. To these they add 3 of their own. One of the patients was a newborn infant, the second a child of 15 months and the third a 53 year old man. The tumors of this special group were congenital and solitary; each was situated in the myocardium and increased slowly in size with the growth of the heart. They were not encapsulated. They were composed of fibrous and elastic tissue, the latter increasing progressively with the age of the lesion, a fact which is interpreted as evidence of adaptation to cardiac function. They were neither inflammatory nor neoplastic but were congenital maldevelopments. A lesion of the type described is termed a fibroelastic hamartia. It is a maldevelopment of the interstitial tissue of the myocardium in the same way that the cardiac rhabdomyoma is a maldevelopment of the muscle. O. T. SCHULTZ.

FORMATION OF FIBRILS IN CULTURES OF THE JENSEN RAT SARCOMA. MARYAN ROZYNEK, Virchows Arch. f. path. Anat. **302**:405, 1938.

This is an experimental attempt to answer the question: Do the immature cells of rapidly growing tumors permanently lose their power of differentiation, or is this power merely held in abeyance? When the Jensen rat sarcoma, which in the host is very cellular and shows no formation of fibrils, was grown in vitro, especially under conditions that retarded growth, it showed formation of collagen and elastic fibrils. The fibrils were produced by the sarcoma cells, the process being identical with the formation of fibrils in cultures of normal connective tissue. When fibril-forming explants were reimplanted into rats, the usual cellular, nonfibrillated neoplasm developed. The findings indicate that the property of differentiation is not permanently lost by the sarcoma cell. O. T. SCHULTZ.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

BAXTER L. CRAWFORD, *President*

H. L. RATCLIFFE, *Secretary*

Regular Meeting, March 9, 1939

Laxity as to the Qualifications of an Expert in the Commonwealth of Pennsylvania as Illustrated by Recent Legal Testimony. HERBERT LUND.

This is a report of legal testimony given by a pharmacist-chemist who for years has practiced certain important branches of hematology and serology. It points out a weakness in our judicial system. A recent murder trial forms the basis of the report (Commonwealth of Pennsylvania vs. James A. Reilly and others in the court of Oyer and Terminer of Fayette County, Pa.; 11/67; December term, 1936). The pharmacist-chemist testified for the Commonwealth of Pennsylvania as to the nature of certain dry stains. According to his report, he identified the stains as blood and determined the groups. He used a physiologically unsound method in determining the groups of the dried blood, attempting to recover the erythrocytes in saline solution and agglutinate them, a method similar to that usually used in typing fresh blood. Such an application of the method is not advocated by any textbook or authority. I questioned his results and demonstrated a fallacy of the method to the jury. The point was made that unless the dry red cells were fixed and insoluble they would hemolyze when aqueous solutions were added (Guthrie, C. C.: *Am. J. Physiol.* **8**:441, 1903), and no cells would be left for the agglutination reactions. The pharmacist-chemist when subjected to a coached cross examination displayed a surprising ignorance of elementary hematology and serology. He admitted that he had never examined blood microscopically except under low magnification (100 diameters). He admitted that he had never identified leukocytes "because they are so in the minority, it is a mighty hard thing to find microscopically." He knew of no books that described leukocytes. He did not know that stains could be used to bring out differences in blood cells. He repeatedly stated and affirmed that erythrocytes after months of drying were still alive "in every sense of the word," because if they were dead they would hemolyze and could no longer be seen. He consistently and repeatedly described erythrocytes as having nuclei (in contrast to leukocytes) and as being larger than leukocytes. He did not know of the concave surfaces of the erythrocytes.

Grouping of dried blood stains is one of the most difficult branches of serology, yet the pharmacist-chemist undertook this work with practically no elementary experience. He admitted that the only fresh blood he had ever typed was his own. In spite of this inadequate training he had, on many occasions, testified in the courts of Pennsylvania and nearby states as to the groups of dried blood stains.

This illustrates the shortcomings of a system in which the qualifications of experts are judged by laymen. It is apparent that laymen cannot evaluate an expert's technical knowledge or the adequacy of his methods but must base their decisions on his age, years of experience, college degrees, official titles and personal appearance. Laymen do not appreciate the limitations of an expert's field, and there is a tendency to enlarge his scope. In this case, serology was

considered well within the field of a general chemist. Cross examination under ordinary circumstances is inadequate. In many cases the object of cross examination is to embarass the witness and make him lose prestige rather than to evaluate his ability. Usually, incorrect answers are not recognized by the judge and jury. When disputed, the witness' "opinion as an expert" makes a simple question appear to be at best a highly controversial point. Even when the opinion is contested by one qualified to do so, the matter simmers down to one man's word against another's.

The Need for Improvement in Medicolegal Investigation. T. A. GONZALES, Chief Medical Examiner, New York.

Of the various branches of medical science, forensic medicine has received the least attention. Few universities have provided in their curriculums courses of instruction in this science. The opportunity to acquire experience in the subject by practical application is presented only in the larger cities, where sufficient material may be available. As a result, the lack of trained experts in this field may be responsible in some degree for the inferior quality of the medicolegal investigations in the majority of jurisdictions in this country.

Other factors are the tenacious retention of the coroner system with its many inadequacies and the failure in some communities to recognize that accurate establishment of the causes of death and other medical facts in cases of violent, suspicious or sudden unexpected death is purely a medical function and should not be relegated to lay coroners' juries.

The coroner's being an elected official (often a layman) with a short tenure of office and the combination of medical and magisterial functions are the fundamental weaknesses of the coroner system. Where efforts have been made to divorce the medical from the judicial functions, the necessity for trained medical investigators has become apparent.

The establishment of the medical examiner system in the city of New York in 1918 created a sound basis for future development. In 1927 Essex County (Newark), N. J., adopted it. This system is purely investigatory, and its primary function is to establish accurately the causes of death and other medical facts in cases coming under its jurisdiction. The law which created the New York office of medical examiner requires that the personnel shall be selected from the Classified Civil Service, with a permanent tenure of office. It specifically indicates the methods to be pursued in investigations, particularly in regard to the visit to the scene and the investigation of the circumstances of the death, the jurisdiction of the medical examiner over the body, the form of report and the power of the medical examiner to perform a necropsy when in his opinion a necropsy is necessary. It also empowers him to take possession of any object found at the scene which in his opinion is necessary for the investigation.

Laboratory facilities are provided for toxicologic, chemical, histologic, bacteriologic and serologic studies. While the necropsy remains the essential procedure, there is a certain percentage of cases in which it fails to reveal the cause of death, and it is then that the laboratory investigations become important. The necessity to determine the presence of alcohol and other drugs in cases of death by violence, unexplained death or suicide by poison is well established. For this purpose a toxicologic laboratory is indispensable. The establishment of the blood groups should be a routine procedure in explaining a violent death, and the examination of blood spots found at the scene or on the clothing of the victim or the suspect when a homicide has occurred is also essential in some cases. Postmortem bacteriologic investigations are often the means of clearing up cases in which obscure types of infections are a factor.

To summarize, the modern medicolegal system should be an impartial fact-finding medical organization, administered by trained medicolegal pathologists and laboratory experts. Adequate facilities should be provided for the practical application of appropriate branches of medical and other sciences to the investiga-

tions. The accurate establishment of causes of death and other medical facts which may be useful for presentation in court should be the primary consideration. In such an organization magisterial functions have no place; they should be relegated to the judiciary, where they belong, i. e., to the magistrates' court, the grand jury and the criminal courts.

The Coroner and the Medical Examiner. R. P. CUSTER.

"If there is virtue in antiquity, the coroner's office must have it" (Schultz, O. T., and Morgan, E. M.: The Coroner and the Medical Examiner, with a Supplement on Medical Testimony by E. M. Morgan, Bulletin 64, National Research Council, Committee on Medico-Legal Problems, Washington, D. C., National Research Council, 1928). It is definitely known to have existed in England as far back as 1194, the coroner maintaining an important status as a representative of the crown and a conservator of the peace, bringing criminals to justice and acting in default of the sheriff. His most significant duty then, as now, was to hold inquests on the bodies of those supposed to have died by violence, by accident or in prison. His qualifications were knighthood, residence in the county and property.

Transplantation of the office to our system of government was natural enough; the duties, however, were confined largely to the investigation of deaths occurring under unusual circumstances, especially those in connection with which violence or criminality was suspected. Briefly, the coroner must decide, after a survey of the case, whether an inquest shall be held; if an inquest is to be held, usually with the aid of a jury and evidence submitted by his deputies and medical assistants, he must decide not only the cause of death but also what person, if anybody, is responsible and must initiate steps for the apprehension and indictment of any one accused. Thus, the coroner's endeavor assumes both the medical and the judicial aspects of criminal investigation, including study of the corpus delicti and the hearing of testimony of witnesses. Unfortunately, the coroner in this country was made an elective officer of the county, subject to the vagaries of partisan politics; he is none too well paid and usually is hampered by the fact that the budget is too niggardly for the maintenance of a competent staff of well trained, properly equipped assistants; oft-times he is forced to employ meritous vote getters. Although his duties are both medical and judicial, he is rarely required to be a physician or a lawyer, and seldom is. In Britain, where the office of coroner is still appointive, as recently as 1926 previous inadequacies in this respect were partially corrected by an amendment stating that the incumbent must be a "barrister, solicitor, or legally qualified medical practitioner of not less than 5 years' standing in his profession"; his tenure of office is permanent, with provision for pension. But even with these improvements the English system falls far short of that of the European continent, where institutes of forensic medicine assume major standing in university structure and where crime detection has attained high repute.

As regards the coroner's physician, he is rarely a career pathologist and practically never devotes full time to the work; he is thereby definitely handicapped in the duties expected of him. Nor can any blame be attached to him. It would be utterly ridiculous to ask a physician to give up his established practice for an inadequately salaried office, the tenure of which depends on the uncontrolled will of the coroner and changes in party administration. The coroner's physician rarely, if ever, is an eye witness to the actual scene of death with body and environs undisturbed. Consequently he must sometimes deal with what is essentially a medical problem on data furnished by the police or a deputy coroner.

In the main, facilities for a complete medical survey of a case are woefully lacking. The equipment for postmortem examinations is usually mediocre, not uncommonly of the worst. How often is the coroner's physician handicapped, even at an impasse, in the final intelligent interpretation of his gross findings through lack of microscopic examinations of tissues and bacteriologic and chemical studies?

In sharp contrast to all of this, Dr. Gonzales has given an impressive picture of a well organized, effectively functioning medical examiner's office, still undermanned, however, and with too limited a budget to care completely for the tremendous duties imposed by a metropolitan population. Again, the system affects only the city of New York, rather than being statewide as it is in Massachusetts. A weakness in the system in Massachusetts lies in the fact that outside of Suffolk County (Boston) the examiners are largely physicians, not pathologists, so that at least some of the work and records lack uniformity and completeness.

The obvious advantages of such a system have been demonstrated, and the analysis by Schultz and Morgan, from which much of my material has been drawn, offers an utter condemnation of the old and outmoded coroner's office. It is apparent that the adoption of the medical examiner system would: (*a*) withdraw the office from politics; (*b*) unify and vastly improve the investigation of death occurring under unusual circumstances, in close harmony with police and legal offices; (*c*) force improvement in laboratory facilities in rural districts and maintain a check on the standards of autopsy studies in all hospitals; (*d*) augment the teaching of forensic medicine in medical and law schools, and (*e*) encourage research and publication in forensic medicine.

It should be emphasized finally that salaries must be adequate and that graded increases in salary and rank must be established to furnish incentive for men of high caliber to accept the responsible positions and for young men of equal quality to enter this branch of medicine as a career. Even so, the ultimate cost to state and county would show little if any increase, more probably a decrease.

NEW ENGLAND PATHOLOGICAL SOCIETY

SIDNEY FARBER, *President*

BENJAMIN CASTLEMAN, *Secretary*

Regular Monthly Meeting, Oct. 19, 1939

Effect of Irradiation on the Blood. CHARLES E. DUNLAP.

The circulating blood cells are quite resistant to destruction in vitro by radiation. It is probable therefore that changes in the peripheral blood picture depend in large part on damage to blood-forming organs. The major damage affects tissues lying within the field of irradiation, but lesser degrees of injury occur in distant bone marrow and lymphoid organs.

The lymphocytes, polymorphonuclear leukocytes and red cells do not show parallel responses to any one technic of irradiation. The resulting blood picture represents a balance between the vulnerability of each cell type and its powers of recovery. Lymphoid organs are highly radiosensitive. They show early and marked damage after small doses but they also recover rapidly from injury. Granulopoietic foci in the bone marrow are moderately radiosensitive, but they recover a little more slowly and less completely than lymphoid organs. Erythropoietic foci are fairly radioresistant, but, once injured, they show poor powers of recovery.

After a single therapeutic exposure to a fairly heavy dose of radiation, a decrease in the number of circulating lymphocytes can be detected within an hour. The decrease continues for about three days and is followed by recovery to normal or above in thirty to sixty days. The polymorphonuclear leukocytes show a transient increase in number, reaching a maximum about twelve hours after exposure. This probably represents mobilization rather than new formation of cells. Subsequently the polymorphonuclear cells fall below normal, reaching a

minimum value some six days after exposure and then recovering to normal a little more slowly than the lymphocytes. The red cells are seldom affected by a single therapeutic exposure but may show a slight decrease in number beginning a week or ten days after exposure. During the first week or two after treatment a few degenerating white cells may be found in circulation. There is a slight shift to the left in the Arneth count, and in some cases mild reticulocytosis occurs. The behavior of the basophils, eosinophils and monocytes has not been adequately studied, but they probably follow the neutrophilic granulocytes. The blood platelets probably increase after small doses and certainly decrease after massive doses.

Patients seldom show serious damage of blood by radiation unless subjected to treatment with massive doses or to prolonged treatment. Radiologists and their technicians, through slight carelessness, may expose themselves to repeated small doses of radiation and suffer significant injury of the blood. Massive overexposure damages cells of all types and leads to death from agranulocytosis or anemia. Slight or moderate chronic overexposure results in leukopenia with relative or absolute lymphocytosis, often associated with anemia. Radiation anemias are ordinarily characterized by parallel reductions of red cells and hemoglobin without change in the appearance of the remaining blood cells. In a considerable number of cases, however, one notes an elevated color index with anisocytosis and poikilocytosis, an occasional slight increase in blood bilirubin and rare megalocytes. Associated with leukopenia and relative lymphocytosis, this picture may be difficult to distinguish from pernicious anemia.

Occasionally radiologic workers show bizarre regenerative states, probably resulting from overcompensation of the blood-forming organs for repeated slight injury. Various degrees of lymphocytosis, monocytosis, eosinophilia and erythrocytosis have been described. Since 1911 reports of 28 instances of leukemia occurring in radiologic workers have appeared. Both lymphatic and myelogenous leukemia have followed exposure to roentgen rays as well as that to radioactive compounds. Some doubt exists as to the etiologic role of radiation in these cases even though several workers have shown that the incidence of spontaneous leukemia in experimental animals can be greatly increased by repeated small doses of radiation.

The treatment for abnormalities of the blood resulting from irradiation is immediate removal of the subject from further exposure. Recovery under these conditions is usually uneventful. Radiologic workers should have periodic complete examinations of their blood. Leukopenia with relative lymphocytosis is evidence of inadequate protection, and anemia indicates that severe damage of the blood has already taken place.

DISCUSSION

SHIELDS WARREN: There is little that I can add to the summary that Dr. Dunlap has given so clearly, but there are a few points that I should like to stress a little more than he has. In the first place, it is generally the responsibility of the pathologist to keep an eye on the hematologic work of the hospital, and it is consequently the responsibility of the pathologist to see that the hospital radiologists and technicians do not get into difficulty. I am afraid pathologists do not accept that responsibility as seriously as they should. While the amount of 0.2 roentgen has been stated as a probably safe amount, it has been stated arbitrarily. There is no exact knowledge on the subject, and, moreover, it is hard for one working with roentgen rays or radium to get average exposure. Consequently I feel that the pathologist will be doing a real service to his medical colleagues in the fields of roentgen radiation and radium if he keeps a careful eye on their blood and notes any of these changes that may take place. As Dr. Dunlap has pointed out, recovery is usual, and given half a chance, say six weeks out in the sun, it is amazing how completely the normal blood picture may come back.

KENNETH LIVINGSTONE: In order to gain an impression of the changes in the white cell picture following the application of roentgen rays of high voltage

over large fields, we have reviewed 12 cases in which treatment of this type was used during the past six months at the Collis P. Huntington Hospital. The million volt machine was used, the patients receiving from 800 to 9,000 roentgens over a 30 by 30 cm. field. No patient showed any indication that anemia was developing during treatment. All showed definite leukopenia. This reduction affected all the white cell forms except in 2 patients who had malignant lymphoma with very low lymphocyte counts before treatment; these 2 patients showed no change in the lymphocytes. Compared on the basis of relative change in total number of cells, the lymphocytes were the most labile of the white cell elements; the mononuclears showed moderate sensitivity, and the polymorphonuclear series was most resistant during the period of treatment. In several cases in which the blood was studied three to six weeks after completion of the course of treatment, it gave evidence of continued depression of the polymorphonuclear series although the lymphocytes and mononuclears were apparently in the rebound phase described by Dr. Dunlap. In 8 of the cases young forms of the polymorphonuclear series either appeared or increased in number during treatment. The impressions gained from these few cases are that the lymphocytes are the most labile of the white cells—their response to injury is quantitatively marked—but that their recovery is rapid; that the polymorphonuclear series is relatively resistant to injury but that the effect of injury is more prolonged, and that the mononuclear series is intermediate in reaction and less consistent than the other two forms.

TRACY B. MALLORY: Since Dr. Warren has raised the question in regard to radiologists, I think I might mention the slight experience we have had at the Massachusetts General Hospital. During the last two years my associates and I have been examining the staff at frequent intervals. I think it is probable that the figures just heard here are in general those for persons who have had one or more heavy treatments, and it is quite likely that the picture resulting from chronic minimal exposure may turn out different from that in the x-ray department. At the Massachusetts General Hospital a considerable percentage of the doctors and technicians have white cell counts running distinctly above normal limits, as high as 15,000 in some instances. One patient has a red cell count of 6,000,000. In other words, the element here seems to be that of stimulation rather than depression, and, since the parallelism of benzene poisoning was brought out, it is also worth remembering that in a considerable percentage of cases of mild exposure to benzene increase rather than decrease in both white and red cell counts was noted.

GEORGE WATT: What could be considered a good interval, or how many examinations should a person exposed to roentgen rays have, to be adequately protected?

SHIELDS WARREN: Examination about once a month should enable one to detect any early change in the blood.

WILLIAM FREEMAN: Given a patient who has moderate anemia established from roentgen radiation, is any specific therapy effective?

CHARLES E. DUNLAP: I do not believe there is any specific treatment for the anemia. One merely stops the exposure to radiation until recovery has proved adequate.

SIDNEY FARBER: Has there been any correlation between age and radiation effect?

CHARLES E. DUNLAP: A few experiments show that younger animals are a great deal more sensitive than older animals. A pregnant rabbit irradiated two days before delivery and two days after delivery will die of anemia. Young rats irradiated shortly after birth will have more profound anemia in a shorter time than older rabbits. I do not know of any work on human beings.

Fat Embolism. LORNE M. GRAY.

Fat embolism was produced in rabbits by intravenous injections of fat extracted from the long bones of rabbits. The fatal dose was found to be between 0.8 cc. and 1.1 cc. per kilogram of body weight. One series of rabbits was given increasing doses of fat (0.1 to 0.8 cc. per kilogram); the animals were killed in twenty-four hours, and the total fat was extracted from the lungs. Another series was given sublethal doses of 0.5 cc. per kilogram; the rabbits were killed in one to seven days, and the total fat was extracted from the lungs. This formed a basis for the study of the distribution of fat in the body and its disappearance from the lungs. This was compared with the histologic picture in the lungs.

Various emulsifying agents were used, but these failed to effect recovery in rabbits given injections of fat.

One series of rabbits, however, when started on small doses and given gradually increasing doses over a period of three to four weeks became tolerant to fat, so that they showed no ill effects on receiving in single successive doses amounts which ordinarily would cause their death. This tolerance to fat has been reported by two other groups of investigators but remains unexplained.

The correlation of many experimental results to observations in patients with fatal fat embolism is conflicting. The histologic picture is very similar, but the fatal human dose and the certain origin of this embolic fat are still under dispute.

DISCUSSION

BENJAMIN CASTLEMAN: Were histologic sections of the lungs made and, if so, was there anything that suggested lipoid pneumonia?

LORNE M. GRAY: The question of lipoid pneumonia is an interesting one. The usual reaction to fat embolism in the rabbit is a few polymorphonuclears and congestion of blood vessels in the lung—hardly comparable to human lipoid pneumonia, in which mononuclear fat-laden phagocytes predominate. However, in animals that have lived a month after the last injection there is some fat in the alveoli. In some of the human beings whose cases are reported in the literature large areas of hemorrhage were present around the fat, but I have never seen that to any extent in experimental animals.

DONALD A. NICKERSON: Is there any correlation between the amount of tissue in the lungs containing fat emboli and fatality?

LORNE M. GRAY: The distribution of fat in the lungs is more or less patchy in the experimental animal and in man. Fat is present in the capillaries, arterioles and alveolar spaces; but the longer those animals which have received sublethal doses live before they are killed the less the fat that is seen in capillaries. The spread appears to be from capillaries to arterioles and then into alveolar spaces.

Relation of Chronic Mastitis to Carcinoma. SHIELDS WARREN.

Although chronic mastitis has been recognized as an entity, and although its relationship to carcinoma of the breast has been under discussion for over a hundred years, there have not been made available as yet any generally accepted data that would permit the surgeon to know what chance of development of carcinoma he requires his patient to take when he leaves a focus of chronic mastitis in the breast. Most studies have been carried out on the basis of finding evidence of coexisting carcinoma and mastitis in the surgically removed material. Two difficulties are encountered with this method of investigation: First, any appreciable degree of association might be coincidence. Second, the mastitic changes might be the result rather than the precursor of the carcinoma.

Since the practical question that the pathologist and the surgeon have to face is what will happen to the breast showing chronic mastitis, it seemed that the best way of dealing with the subject was to follow an appreciable group of cases for a long enough period of time to gain a fair idea as to what the ultimate behavior of such a breast might be.

The present study was undertaken from that standpoint. It is based on 1,206 cases of various types of diseases of the breast which were followed for five years or longer. The exceptions to this follow-up period were the cases in which carcinoma developed in less than five years after the first operation for a benign lesion of the breast.

In order to test further the statistical validity of the results and to insure an adequate variety of the samples selected, 602 cases were taken from the files of the Toronto General Hospital in Ontario and 604 from those of various Boston hospitals. The Boston group was further divided into two samples. These three groups checked with one another closely. The vast majority of the cases were cases of chronic mastitis and chronic cystic mastitis, but there were included 21 cases of adenoma and 70 cases of adenocystoma. Among the latter, there were 7 cases of carcinoma, an incidence of 8 per cent.

In the group of chronic mastitis and chronic cystic mastitis there were 35 cases (a percentage of 3.4) in which cancer developed either in the previously involved breast or in the opposite breast. When the incidence is calculated on the basis of the number of years of exposure and contrasted with the attack rate rather than the mortality rate, the occurrence of cancer is found to be 0.37 per hundred as against a calculated rate of 0.03 per hundred in the entire female population of Massachusetts in 1930.

The average duration of the follow-up study was nine years, and the age distribution of the cancers was such that approximately one half the patients were under 50 and one half over 50 years of age.

One of the most difficult problems is that of determining what the morbidity rate of cancer of the breast in the female population is. I have assumed that the annual mortality rate multiplied by 2 would equal the annual attack rate on the basis that the number of patients cured of cancer of the breast and the number of patients whose cancer was never diagnosed would perhaps equal the number of patients dying from the disease. This is probably an excessively high allowance, but it seems wise to work cautiously in this field and overestimate rather than underestimate the morbidity rate of cancer in the general population.

When one calculates from our material the age-specific cancer rates and contrasts them with those of the Massachusetts population and of Canada, two things stand out in startling fashion. The first is the marked predominance of cancer in the group with antecedent pathologic changes in the breast. The second is that there is nearly 12 times as much cancer of the breast developing in women from 30 to 49 years of age in the previously diseased group as in the general population and only 2.5 times as much as in those over 50 years of age. In other words, the fact of antecedent pathologic changes in the breast has most weight when it concerns a woman in the earlier of these age groups.

When one turns to the histologic observations in those cases in which mastitis was followed by carcinoma, the results are most discouraging. There is no form of lesion singled out, but instead one finds practically any type of lesion being followed by cancer of the breast. In general, intraductal proliferation of the epithelium of the ducts appears to be the most important suggestion of subsequent malignancy. The large columnar acidophilic cells that are occasionally seen lining cysts or projecting in papillary formation seem to be relatively rarely associated with the development of cancer. It is not unnatural that there should be a relatively high rate for the development of cancer from true intraductal papillomas since at times it is possible to see infiltrating carcinoma spreading from the base of the lesion in ordinary histologic material. However, no form of abnormal change in the epithelial elements of the breast seems to be safe. It is my impression that the development of chronic mastitis, chronic cystic mastitis, adenoma or cystadenoma is an expression of an undue ability of the mammary epithelium which predisposes to the development of carcinoma and that no one type of lesion can be regarded as definitely precancerous, on the one hand, or definitely dissociated from subsequent development of carcinoma, on the other hand.

From the practical standpoint, one is faced with the fact that in an average follow-up period of ten years there were 42 cases of cancer of the breast against a calculated expectancy—every conservative adjustment being used—of 13 cases. This incidence of mammary cancer following a preexisting lesion of the breast is not sufficiently great to demand bilateral mastectomy as a preventive measure. The incidence is, however, sufficiently great, particularly among women of the younger age groups, to move one to consider them as constituting a special risk group that should be followed with great care, probably at least at six month intervals, with resort to amputation when any suspicious change in the breast is noted. Lest this last suggestion seem too radical, I add that if one combines the number of patients who have had cancer develop with the number of patients who have had recurrences of their benign mammary lesions necessitating operation, the percentage is strikingly high.

DISCUSSION

CHANNING C. SIMMONS: I have been much interested in hearing Dr. Warren's paper and comparing it with work that Dr. R. B. Greenough and I did several years ago. In 1914 we were struck with the number of cases of cystic mastitis in which malignant disease developed later. We studied a series of 83 cases of cystic disease of the breast in which partial removal was performed. In 4, or 4.8 per cent, cancer developed in the breast which had been partially removed; in 8 per cent further cystic disease developed requiring amputation; in 5 per cent cystic disease developed in the other breast, requiring amputation of that breast as well as of the first breast, whereas 66 per cent were what might be called "successful cases." Because of the incidence of cancer (4.8 per cent) I now advise simple amputation for cystic disease rather than partial resection. Occasionally I am inveigled into doing partial removal and often I am sorry afterward. As regards the second breast, I have no figures, but in many instances I have had to remove it because of pathologic developments.

IRA T. NATHANSON: Three or four years ago Dr. Claude Welch and I were interested in the age incidence of carcinoma of the breast. The figures accepted up to that time were those of Dr. Pack, of the Memorial Hospital, New York, which are rather contrary to the general belief, probably because he has a distinctly special group of patients, referred to the hospital only for the treatment of cancer. If one compares the incidence of cancer of the breast in a general hospital with that in the general population I think one is able to show that the incidence of cancer in a general hospital rises steadily with age. Accordingly, we looked up the death certificates in Massachusetts from 1928 to 1930 and, correcting for the fact that one third of all patients with cancer never reached the hospital before death, found a distinct rise in the incidence of carcinoma with age. Therefore these figures confirm Dr. Warren's figures based on a logarithmic curve. The group with mazoplasia present a picture very different clinically in our minds from that observed in the group with frank cystic disease. In questioning a good many patients with carcinoma of the breast as to whether they had cyclic activity in that breast we were struck by the absence of this syndrome.

B. EARLE CLARK: One other question I wish Dr. Warren could answer is: What is the incidence of chronic cystic mastitis in the general population? He has pointed out that in a number of cases carcinoma remains undiagnosed, and I think it is fair to assume that there are a number of cases in which chronic cystic mastitis remains undiagnosed, probably much larger than that in which carcinoma remains undiagnosed. Some writers put it as high as 100 per cent, without much specific scientific evidence.

SHIELDS WARREN: I have no way of knowing the incidence of chronic cystic mastitis in the general population. On the basis of female hospital population and from what I have been able to gather from the results of routine physical examinations made by various men, cystic disease is not by any means infrequent. On the other hand, I should think that 100 per cent is a long way from the truth. There are many women with cystic disease who do not have carcinoma. I think

all physicians can do is to work on the basis of the cases they have been able to study and decide what to do for the patients in the light of present knowledge. If in the course of a routine physical examination cystic mastitis is encountered, it should be regarded as important.

B. EARLE CLARK: As regards making a separate group of the cases of chronic cystic mastitis, my opinion is that one can frequently find all the histologic features of both groups within the same breast and that a small portion of breast removed for pathologic examination is not a true sample of the whole breast. There may be epithelial hyperplasia in one part and fibrosis in another.

SHIELDS WARREN: I am in complete accord with this point of view. Although I first separated the types of breast changes in this study, my results were about the same, so I lumped them all together as one. In the study of chronic mastitis and chronic cystic mastitis there is a tendency to find all gradations.

ROBERT FIENBERG: There is one point I should like to have Dr. Warren clear up in relation to the high percentage of carcinoma following cystic mastitis in the lower age groups, which I attribute to hormonal stimulation of the breast. In the general curve, however, showing an increase in the incidence of carcinoma of the breast in older age groups, I am struck by the fact that, especially in the very old, in whom there is a very high incidence of cancer of the breast, there has been no hormonal stimulation for a long time, up to thirty-five to forty years. This long period is inconsistent if the cancer is due to hormonal stimulation. In other words, it seems as if the mechanization of cancer is different in the younger groups with cystic mastitis than in the older groups.

SHIELDS WARREN: I should hesitate to claim that chronic mastitis and chronic cystic mastitis are important factors in carcinoma of the breast. All physicians can do on the basis of the data they now have is to deal with these lesions of the breast in the light of those with which they have come in contact. The important thing is to point out that the incidence of carcinoma in any organ, not only in the breast but in the skin, the prostate and the stomach, tends to increase markedly with increasing age. Now a slight hint as to the explanation of this has been thrown out by some of the hormonal work, which suggests that atrophic epithelium is much more likely to respond abnormally with stimulation than epithelium which has not atrophied. Whether that is going to be borne out in the future I cannot say. I do not want you to carry away the idea that any appreciable volume of carcinoma of the breast comes from chronic cystic mastitis. All I am prepared to say is that women with chronic cystic mastitis and those with papillary cyst adenoma represent a group of women that have a special hazard and so deserve particularly careful attention.

Book Reviews

The General Tissue and Humoral Response to an Avirulent Tubercle Bacillus Including Growth Characteristics of the Organism. Sol Roy Rosenthal, M.D., Ph.D., Associate in Bacteriology and Public Health. Joint Contribution from the Tice Laboratories of the City of Chicago Municipal Tuberculosis Sanitarium and the College of Medicine of the University of Illinois. Illinois Medical and Dental Monographs, Vol. II, No. 2. Paper. Pp. 184, with 80 illustrations. Price $2.50. Urbana: University of Illinois Press, 1938.

The family of acid-fast bacilli still offers many questions to the investigator. This book represents the efforts of its author to elucidate some of the unknown factors of one member of this family, namely BCG. The author has studied the BCG micro-organism from numerous standpoints and has embellished his record with meticulous detail. The study embraces the effect of fats when incorporated into mediums on bacillary growth. It takes into account the response of the several tissues and organs of the guinea pig's body to infection by various accepted routes, namely, intravenous, intradermal and oral. It records the tissue reaction to injection of fractions of the tubercle bacillus and discusses the so-called submicroscopic forms and their effect on the reticuloendothelial system. It indicates that the life cycle is much like that which Kahn has described for the tubercle bacillus.

The book represents a prodigious amount of work. This in places seems of high order; in other places it gives the impression of lack of discrimination, lack of discretion in listing detail and lack of *critique*. In some areas the detail appears to be laboratory notes almost verbatim. The author presents a great deal of factual information as to the presence of acid-fast bacilli in various organs of the body remote from the site of inoculation, as well as much factual matter on the histologic response in these areas. He appears, at the same time, to have no difficulty in identifying readily all types of cells and cellular structure involved in the areas of reaction. The microscopic studies have been carried out in great detail in practically all the organs of the body and at many periods of time after inoculation, from a few minutes to longer than a year. Blood responses, especially the monocyte-lymphocyte ratio, have received considerable attention.

The contention that the inoculation of bacilli produces prompt effects in remote parts of the body through its influence on the entire reticuloendothelial system is engaging in some respects. It is stated that the cellular response is prompt in remote parts of the body—presumably the reaction is to "submicroscopic" forms of the tubercle bacillus. Organs showing the reaction are culture negative for the most part, but transfer through a series of animals produces sensitiveness to tuberculin and tuberculous disease such as is claimed for the filtrable forms of the bacillus. The submicroscopic forms appear by inference to be bacillary granules or something near filtrable forms. The case for transfer of infectious matter through animals by several passages is suggestive but not convincing.

The book contains numerous, and in places confusing, lapses in sentence structure and shows poor proof reading. Each chapter carries a bibliography.

One is glad to have this book in one's library for reference, yet one cannot but feel that elimination of a great deal of unrelated factual matter might have made the book more attractive and more valuable.

La ponction sternale. Procédé de diagnostic cytologique. P. Emile-Weil, Médicin des Hôpitaux de Paris, and Suzanne Perlès, Chef de laboratoire a l'Hôpital Tenton. Paper. Pp. 183, with 25 illustrations and 9 colored charts. Price 75 francs. Paris: Masson & Cie, 1938.

This book reviews the field of application of sternal puncture, pointing out its advantages but also sources of error. The authors in 1936 published a book on

splenic puncture, and Emile Weil is also the author of a volume on diseases of the blood. The present work is the outcome of years of study of material obtained by puncture of the hemopoietic centers (spleen, liver and sternum). The material is divided in two main parts. The first deals with the technic of the puncture, preparation of smears and staining. The cytology of the bone marrow is presented briefly but clearly with the help of some excellent colored drawings of marrow cells. The proportions of the cells in the normal marrow are tabulated in the form of a so-called normal myelogram. The relations of the different cells to one another are then expressed in the form of indexes. The second part deals with the findings in disease. The different leukemias, the leukemoid conditions, the neoplastic primary and metastatic growths of the marrow, the anemias, the polycythemias, the infectious diseases which influence the marrow, some diseases of the liver and spleen (kala azar, malaria, Gaucher's disease and finally Hodgkin's lymphogranuloma and infectious mononucleosis are discussed.

For every disease which presents characteristic changes in the marrow, the cytologic data are tabulated in the form of a myelogram. The differences in the appearance of the cells in the same stage of differentiation in the marrow and in the circulating blood are emphasized, particularly with regard to plasma cells. The concept of the myelogenous origin of the last cell and the implications with regard to the genesis of the so-called plasma cell myeloma are interesting. The sternal puncture is, according to the authors, indispensable for the proper diagnosis and evaluation of the anemias; there, as well as in other conditions, it is essential for the prognosis, for the follow-up and for the control of therapy and as an early indicator of relapses. A good case is made in favor of the study of tissue obtained by puncture of the spleen and occasionally of the liver, in addition to that obtained by sternal puncture. In these relatively early years of sternal puncture the present monograph will serve a good purpose. It radiates an enthusiasm which cannot fail to be transmitted to the reader. It might have been better still if the shortcomings of the method had been stressed a little more adequately. They are known to those who have practiced it critically but are sometimes not sufficiently appreciated by others. Such reserves as to what may be expected would save considerable disappointment. The book can be warmly recommended to all interested in hematology. It has 158 references.

La mort des brûles. Étude expérimentale. L. Christophe. Foreword by L. Binet. Paper. Pages 93, with 20 illustrations and 20 tables. Price 40 francs. Paris: Masson & Cie, 1939.

Following a brief introduction comes a review of the literature on the causes of death resulting from burns. Five chapters deal with the clinical picture and death as due to (1) loss of function of the skin, (2) circulatory disturbances, (3) nervous shock, (4) circulatory shock and (5) intoxications. The author then presents the results of his own investigations, in which he was concerned with death occurring several days after a burn and not with death occurring within a shorter period. The work was done on dogs. Some were burned by means of a Bunsen burner and some by scalding with hot water. Some dogs were not burned but were perfused through the burned limbs of other dogs. Long columns of chemical changes in the blood are recorded. These investigations included also determinations of hemoglobin, of blood and plasma volume and of the sedimentation rate. The results indicate that during the first hours after a burn toxic substances are formed. These circulate in the blood and damage certain histologically demonstrable centers in the anterior part of the hypothalamus, which in turn leads to nephritis characterized by lowering of the chlorides and by elevation of the nonprotein nitrogenous substances. The typical changes in the blood and the occasional gastroduodenal ulcerations are due to the same central lesion. A 7 page bibliography is appended. It is obviously incomplete. Several names are misspelled. The monograph is of considerable practical significance. The therapy must be centered on the first few hours following the burn and should aim at a fixation of the toxic substances in the skin. The lowering of the

chlorides of the blood suggests the importance of the introduction of sodium chloride. The anatomic changes in the central nervous system and in the kidneys are illustrated by 16 photomicrographs.

Brucellosis in Man and Animals. I. Forest Huddleson, D.V.M., M.S., Ph.D., Research Professor in Bacteriology, Michigan State College. Contributor Authors: A. V. Hardy, M.S., M.D., Dr.P.H., Associate Professor of Epidemiology, DeLamar Institute of Public Health, Columbia University Medical School; Consultant, U. S. Public Health Service. J. E. Debono, M.D., M.R., C.P., Professor of Pharmacology and Therapeutics, Royal University of Malta. Ward Giltner, D.V.M., M.S., Dr.P.H., Dean of Veterinary Division and Professor of Bacteriology, Michigan State College. Cloth. Pp. 339, with 40 figures. Price $3.50. New York: The Commonwealth Fund, 1939.

This book is a revised and expanded edition of "Brucella Infections in Animals and Man" by the same author, published in 1934. The latter was devoted primarily to a description of laboratory methods for the study of brucellosis. The object of the new book is to meet the needs for a more comprehensive presentation of the growing knowledge of the disease, which Charles Nicolle regarded as "a disease of the future." The scope of the book, which is clearly written and nicely printed, is best indicated by an outline of the contents. The three first chapters deal with Brucella—the general characteristics of the genus, the methods for its isolation and the differentiation of the species. Then comes the section on brucellosis in human beings, in four parts: an historical survey, brucellosis in the United States (by A. V. Hardy), brucellosis in Malta (by J. E. Debono) and treatment. The epidemiologic aspects, the clinical manifestations, the diagnosis and the treatment of the disease receive adequate and competent consideration. In the next chapter brucellosis in animals is reviewed as it occurs in cattle, swine, goats, sheep, other mammals and fowls as well as experimentally in guinea pigs. The various laboratory methods for the diagnosis of brucellosis are well described. In the last chapter Ward Giltner discusses the eradication or control of the sources of brucella infection, which "is a problem in animal hygiene and veterinary medicine, and this is fortunate since veterinary medicine is better organized for the control of this disease than human medicine." There is an appendix of illustrative case reports, a select bibliography of 378 items and a good subject and author index. The book will be of value to physicians, veterinarians and laboratory workers. It is the creditable outcome of individual and cooperative study of the problems of brucellosis.

Protozoology. Richard R. Kudo, D.Sc., Associate Professor of Zoology, University of Illinois. Second edition. Cloth. Price $6.50. Pp. 676, with 291 illustrations. Springfield, Ill., and Baltimore, Md.; Charles C. Thomas, Publisher, 1939.

This volume is the second edition of the author's "Handbook of Protozoology." The change of title was made because of the changes and additions to the text, but the purpose remains an attempt to present "introductory information on the common and representative genera of all groups of both free-living and parasitic Protozoa" to advanced college and graduate students of zoology. The introductory chapter deals with the relationship of protozoology to other fields of biology and gives a short history of the subject. Five chapters are concise statements of the general subjects of ecology, morphology, physiology, reproduction and genetics. Most of the book is devoted to the remaining 37 chapters, which form a taxonomic review of the Phylum Protozoa, in which systematics, morphologic aspects and methods of reproduction are considered and developed in detail. Throughout, the book is well written and beautifully illustrated with pen and ink drawings. Although it is designed as a text for beginners, the taxonomic portion is complete enough to serve as a work of reference for protozoologists who are not specialists in the field. No criticism can be given of the description of the parasitic protozoa, but parasitologists and physicians will be disappointed with the summary treatment of parasitism, immunization and related topics.

Books Received

DIVERTICULA AND DIVERCULITIS OF THE INTESTINE: THEIR PATHOLOGY, DIAGNOSIS AND TREATMENT. Harold C. Edwards, M.S., F.R.C.S. (England), Surgeon and Lecturer in Surgery to King's College Hospital, London; Surgeon to the Evelina Hospital for Sick Children, London; Jacksonian Essayist 1932, and late Hunterian Professor, Royal College of Surgeons of England. Foreword by Gordon Gordon-Taylor, O.B.E., M.S., F.R.C.S. Cloth. Pp. 335, with 223 illustrations. Price $8. Baltimore: Williams & Wilkins Company, 1939.

VIRUS AND RICKETTSIAL DISEASES WITH ESPECIAL CONSIDERATION OF THEIR PUBLIC HEALTH SIGNIFICANCE. A SYMPOSIUM HELD AT THE HARVARD SCHOOL OF PUBLIC HEALTH, JUNE 12–JUNE 17, 1939. Cloth. Pp. 907, illustrated. Price $6.50. Cambridge, Mass.: Harvard University Press, 1940.

A TEXTBOOK OF LABORATORY DIAGNOSIS. WITH CLINICAL APPLICATIONS FOR PRACTITIONERS AND STUDENTS. Edwin E. Osgood, M.D., Associate Professor of Medicine and Head of the Division of Experimental Medicine, University of Oregon Medical School. Third edition. Pp. 653, with 37 illustrations. Price $6. Philadelphia: P. Blakiston's Son & Co., 1940.

ANNUAL REPORT OF THE SURGEON GENERAL OF THE PUBLIC HEALTH SERVICE OF THE UNITED STATES FOR THE FISCAL YEAR 1939. Cloth. Pp. 185. Price 75 cents. Washington, D. C.: Superintendent of Documents, Washington, D. C., 1939.

DISEASES OF THE GALLBLADDER AND BILE DUCTS. Waltman Walters, B.S., M.D., M.S. in surgery, Sc.D., F.A.C.S., Head of Section in Division of Surgery, Mayo Clinic; Professor of Surgery, Mayo Foundation (University of Minnesota); and Albert M. Snell, B.S., M.D., M.S. in Medicine, F.A.C.P., Head of Section in Division of Medicine, Mayo Clinic; Professor of Medicine, Mayo Foundation (University of Minnesota). Cloth. Pp. 645, with 342 illustrations. Price $10. Philadelphia: W. B. Saunders Company.